KFRI Research Report No. 424

Evaluation of *Saraca asoca, Kaempferia rotunda,* their substitutes and medicinal preparations with respect to phytochemical and biological properties

N Sasidharan

Jose Padikkala

Kerala Forest Research Institute
An Institution of Kerala State Council for Science, Technology and Environment (KSCSTE)
Peechi-680 653, Thrissur

KFRI Research Report No. 424 **ISBN No. 0970-8103**

Evaluation of *Saraca asoca, Kaempferia rotunda*, their substitutes and medicinal preparations with respect to phytochemical and biological properties

N Sasidharan
Kerala Forest Research Institute, Peechi 680 653, Thrissur, Kerala

Jose Padikkala
Amala Cancer Research Centre, Amala Nagar 680 555, Thrissur, Kerala

Kerala Forest Research Institute
Peechi 680 653, Thrissur
2012

Title of the project	:	KFRI/545/08, Evaluation of *Saraca asoca, Kaempferia rotunda*, their substitutes and medicinal preparations with respect to phytochemical and biological properties
Objectives	:	• Qualitative and quantitative characterization of phytochemicals in *Saraca asoca* and *Kaempferia rotunda* and their substitutes • Preparation of a comparative account of phytochemical profiles of *Saraca asoca* and *Kaempferia rotunda* and other related species. • Evaluation of the general biological properties such as antioxidants, anti-inflammatory properties etc. of 'Asokarishtam' and 'Drakshadi Kashayam' prepared with preferred species as well as substitutes. • The specific biological activities of 'Asokarishtam' prepared with preferred species as well as substitutes by measuring cornification of the uterus epithelium, the estrogenic level and testing the enzymes involved in prostaglandin synthetase
Principal Investigator & Address	:	Dr N Sasidharan Head & Scientist, NTFP Department Kerala Forest Research Institute Peechi, Thrissur 680 653
Co-investigator & Address	:	Dr Jose Padikkala Professor, Amala Cancer Research Centre Amala Nagar 680 555, Thrissur
Research Fellow	:	Shri AP Shahid
Date of commencement	:	June 2008
Date of completion	:	March 2012

CONTENTS

ACKNOWLEDGEMENTS

The guidance and the moral support and above all, the interest shown by Dr. K.V. Sankaran, Director, Kerala Forest Research Institute are acknowledged with due respect. The financial support for the study by the National Medicinal Plants Board (NMPB) and State Medicinal Plants Board (SMPB) is gratefully acknowledged. We express our sincere thanks to Dr. T.D. Babu, Assistant Professor and Dr. C.R. Achuthan, Assistant Professor, Amala Cancer Research Centre, Thrissur for their help in carrying out biological property analyses and preparation of report. The editorial comments from Dr. K.V. Bhat, Dr. M.P. Sujatha and Dr. R. Jayaraj, Kerala Forest Research Institute are gratefully acknowledged. We also place on record our sincere thanks to Mr. AP Shahid, Research Fellow, Kerala Forest Research Institute and Mr. J. Austin Moses, M Pharm Student, Mr. K. Hareesh, M Pharm Student, Mr. P.T. Suhile, M Pharm Student, Miss Salini Sasidharan, Research Fellow, Amala Cancer Research Centre, Thrissur for their help in carrying out laboratory studies. The help rendered by Dr. K.S. Rejithan, Chief Medical Officer, Panchakarma Hospital, Oushadhi, Thrissur, in the preparation of Arishtams is thankfully acknowledged. We also place on record my sincere thanks to Dr. P. Sujanapal for collecting materials and to Shri M.M. Roy for typesetting and layout of the report.

ABSTRACT

Saraca asoca (Asoka) and *Kaempferia rotunda* (Chengazhi) are well-known medicinal plants to treat various human ailments in the Indian traditional systems of medicine. In Ayurveda, the bark of *Saraca asoca* is an important raw drug of 'Asokarishtam', a fermented preparation, generally used to treat uterine menorrhagia. *Kaempferia rotunda* is the prime raw drug of *Drakshadhi decoction* used against fever due to *vata* and *pitta*, vomiting, fainting, burning sensation, fatigue, etc. Due to over exploitation, the natural populations of *Saraca asoca* and *Kaempferia rotunda* have been dwindling over the years in the country. Because of the scarcity, the demand is met largely through substitutes consisting of related or unrelated species. The commonly used alternative for Asoka is *Polyalthia longifolia.* Occasionally, *Kingiodendron pinnatum* and *Cynometra travancorica* are also used. *Lagenandra ovata* and *L. toxicaria* are found to be substituted for *Kaempferia rotunda.* The medicinal properties of these substitutes are not studied well. A species can be treated as a substitute if it can provide similar therapeutic effects like the preferred drug rather than its morphological similarity or phytoconstituents. In this context, the primary focus of the present study was to identify the most suitable alternative for Asoka and Chengazhi. In the present study, we assessed the phytochemical and biological properties of *Saraca asoca* and its substitutes like *Kingiodendron pinnatum, Cynometra beddomei, C. travancorica*, *Humboldtia vahliana* and *H. brunonis* and *Kaempferia rotunda* and its substitutes like *Lagenandra ovata* and *L. toxicaria* and 'Asokarishtam' prepared with preferred species as well as substitutes.

In the comparative phytochemical analysis, the compound profile generated through TLC and HPTLC procedures in *K. pinnatum* and *C. travancorica* were found to be more or less similar to *S. asoca*. But

substitutes of *Kaempferia rotunda* did not produce much detectable similarity. The results obtained in the antioxidant study showed that *Saraca asoca* and its substitutes except *H. vahliana* possess significant antioxidant properties. Among the substitutes of Asoka, *K. pinnatum* was found to be most effective in scavenging superoxide radicals, *H. brunonis* effectively inhibited hydroxyl radicals and *C. travancorica* reduced lipid peroxidation. In the anti-inflammatory study, *K. pinnatum*, *C. travancorica*, *C. beddomei* and *H. brunonis* showed significant activity on acute as well as chronic paw edema in mouse limb. No significant level of antioxidant and anti-inflammatory effects were observed with *Lagenandra ovata* and *L. toxicaria,* substitutes of *Kaempferia rotunda.* So, further works on these plants were not carried out. On estradiol induced keratinization, *K. pinnatum* was found as effective as *S. asoca* in reducing the cornification in rat uterus. The elevated level of estrogen in estradiol administered animals has got significantly reduced by the substitute plants and the maximum reduction was observed with *C. travancorica*. Thus, the results of the present study show that *K. pinnatum* and *C. travancorica* possess phyto-constituents and biological properties similar to *S. asoca*.

Arishtams prepared with *S. asoca* and substitutes were analyzed for their therapeutic efficacy. In the *in vitro* study, individual Arishtams were found to be effective in scavenging free radicals and inhibiting membrane lipid peroxidation. *In vivo*, the antioxidant enzymes such as SOD, catalase and GPX activities and the level of glutathione were elevated in the blood and liver tissues in mice after administration of Arishtams. Among the Arishtams prepared with substitutes, *K. pinnatum* was the most effective. In the carrageenan and formalin induced paw oedema formation, all the prepared Arishtams showed almost similar percentage of reduction. However, the efficacy of Arishtam with *C. travancorica* was comparatively higher. The LPS induced activity of Cox 2 enzyme got diminished on administration of Aristams with *S. asoca* and *K. pinnatum*.

However, less inhibition of cyclooxygenase activity noticed with Arishtams containing *C. travancorica* and *P. longifolia,* suggested the mechanistic basis for their anti- inflammatory effect. In the estradiol induced keratinization analysis on young rats, all the Arishtams except *H. brunonis,* showed significant anti-keratinizing effect. As there are no reports on the specific biological efficacy of *P. longifolia*, on uterine hemorrhagia, Arishtam was also prepared with it for testing. Surprisingly, considerable anti-keratinizing effect on estradiol induced cornification in rat uterus was noticed with the Arishtam containing *P. longifolia*. The efficacy was however, relatively low when compared to other substitutes and *S. asoca*.

The present study suggests that *K. pinnatum* and *C. travancorica* with their phytochemical and biological similarities, are the most suitable plants to be substituted for *S. asoca* in the preparation of Asokarishtam. Interestingly, all the Arishtams prepared including *P longifolia*, showed moderate inhibiting activity in the uterine cornification of rats. The data generated from the study thus provide scientific basis for the use of substitute plants in the preparations of Asokarishtam. The results of the phytochemical and biological studies suggest that *Lagenandra ovata* and *L. toxicaria* cannot be substituted for *Kaempferia rotunda*. Though, promising results were obtained with *K. pinnatum* and *C. travancorica*, both these trees are listed under the threatened category. Hence the exploitation from the wild source is not advisable and efforts are needed for their availability through cultivation. Further, in depth evaluation on the prepared Arishtams is required for their clinical recommendation.

1. INTRODUCTION

Plants, since time immemorial have been used in all civilisations as a source of medicine for the healthcare needs. The widespread use of plants and their preparations are traceable from ancient traditional medical practices in various civilisations in countries like China, India etc. and texts such as the *Vedas*. Even though numerous well accepted modern therapeutics are available today, people still prefer natural products because of the adverse side effects of synthetic products in therapy. Currently, the natural products continue to be widely used through traditional systems of medicine on many accounts and the usage of medicinal plants has been increasing tremendously in Asian as well as Western countries. According to World Health Organisation (WHO), approximately 80 per cent of the population in developing countries depends on traditional medicine, mostly plant drugs, for their primary healthcare needs and it is estimated that approximately 25 per cent of all drugs prescriptions are derived from the plants or plant products. Eventually the modern branch of medicine is realizing that the plants are providing various secondary metabolites with diverse biological properties, which offer a way to control diseases.

Nature has bestowed India with an enormous wealth of medicinal plants and therefore, the country has often been referred to as the "medicinal garden" of the world. The All India Ethnobiological Survey (AIES) carried out by the Ministry of Environment and Forests, revealed that 7,500 plant species are used by 4,635 ethnic communities for healthcare across the country (Dubey *et al.*, 2004). Over the years, the increased demand has led to over-exploitation of medicinal plants. Many have become rare or even depleted from their natural habitats (Gadgil and Guha, 1992; Haeuber, 1993). Moreover, the botanical source of a raw drug is often attributed to one species in most of the Indian systems of medicine. The

continuous extraction of a species will lead to its rarity as well as the biodiversity of the habitats. The scarcity has resulted in adulteration and substitution of raw drugs (Pulok, 2002). Many Indian traditional medicinal preparations are not available in a standardized form due to lack of genuine plant material, quality control, research and development of herbal drugs etc. In order to promote Indian herbal drugs, there is an urgent need to evaluate the therapeutic potentials of the drugs as per WHO guidelines (WHO, 2000).

To overcome the unavailability of the actual plant materials, a variety of approaches have been developed to increase productivity of plants/plant products. Cultivation is an important strategy to overcome the scarcity of medicinal plants. The other measures to increase the production of plant ingredients include generating modified plants through biotechnological tools like plant tissue culture, development of genetically modified plants and induction of secondary metabolites production by applying chemical or fungal elicitors. But the increased application of synthetic fertilizers and pesticides may result in the less production of secondary metabolites and the accumulation of toxic chemicals in plant parts (Patwardhan, 2000). The other general practice is the usage of substitutes. In the absence of the preferred species, the demand is met with substitutes, consisting of related or unrelated species (Rajshekharan, 2002). Substitution is beneficial if it can provide similar therapeutic effect like that of the preferred raw drug. Adulteration of the raw drugs is a serious problem in herbal industry. Hence, correct identification of medicinal plants, chemical constituents and biological property analysis in the phytochemical industry are very important criteria. Some of the laboratories have already initiated analytical studies to determine the concentrations of active ingredients, contamination by pesticides, fertilizers, hormones, mycotoxins and general efficacy of medicinal plants. Substitution of herbs achieved many goals though the basic idea

was to provide similar therapeutic effects as that of original drug (Poornima, 2010). Therefore, the most essential criteria for substitution are the pharmacological activities rather than morphology or phytoconstituents.

Among the threatened medicinal plants, *Saraca asoca* belonging to the subfamily Caesalpinioideae of the family Leguminosae and *Kaempferia rotunda* belonging to the family Zingiberaceae are used in large quantities in Ayurvedic medicines. These are the preferred species in Ayurveda and known as 'Asokam' and 'Chengazhi' (Warrier *et al.,* 1995), respectively. The bark of *Saraca asoca* is an important raw drug in 'Asokaristam' and several other medicinal preparations. The tannins contained in the bark provide the main astringent action for halting excessive menstrual bleeding, and also for bleeding hemorrhoids, bleeding ulcers, and hemorrhagic dysentery. 'Asokarishtam', the fermented formulation containing 'Asokam' is used as a tonic for menorrhagia. The bark is also used to cure biliousness, dyspepsia, dysentery, colic, piles, ulcers, pimples and the alcoholic extract is reported to be active against a wide range of bacteria (Ravikumar and Ved, 2000). Hattori *et al* (1995) have reported that the bark extract of *Saraca asoca* is effective in inhibiting the activity of simplex virus type 1 (HSV-1). Topically, it is used for treating bites, ulcerations, skin discoloration, etc. Moreover, oxytocic efficacy of a phenolic glycoside isolated from this plant has also been reported (Satyavati *et al.*, 1970). Compounds such as tannin, catechol, ketosterol and organic calcium have been isolated from the bark (Biswas and Debnath, 1972; Sarwar, 2002; Sasmal *et al.*, 2012). The pharmaceutical industry in India requires about 5,300 tonnes of bark annually. The consumption of 'Asokam' in the Ayurvedic drug industry in Kerala is about 105 tonnes/year (Sasidharan and Muraleedharan, 2000). Due to over-exploitation, it is almost depleted from its natural habitats. International Union for Conservation of Nature and Natural Resources

(IUCN) has listed this species under the threat category, 'Globally Vulnerable' (Anjan *et al.*, 2004). The scarcity has led to substitution with the bark of other related or unrelated trees. The bark of *Polyalthia longifolia* (Annonaceae) is widely used in place of *Saraca asoca*. *Kingiodendron pinnatum* and *Cynometra travancorica* (Leguminosae) are occasionally used as substitutes. The medicinal properties of these species are not well known.

'Drakshadhi' decoction prepared with the rhizome of 'Chengazhi' is used in Ayurveda against fever due to *vata* and *pitta*, vomiting, fainting, burning sensation, fatigue, etc (Chopra, 1982). The tubers are used to treat abdominal illness and gastric complaints. It is also used in the production of cosmetics. The rhizome and flowers are used to treat injuries and broken bones in traditional Chinese medicine. The annual consumption of *Kaempferia rotunda* in Ayurvedic drug industry in Kerala is about 20,000 kg/year (Sasidharan and Muraleedharan, 2000). Due to scarcity, it is observed that plants like *Lagenandra ovata* and *L. toxicaria* belonging to the family Araceae are used as substitutes.

During the past decades, a large number of compounds like various types of alkaloids, flavonoids, glycosides, terpenes, phytoelexines, coumarins, polyphenols etc., were isolated, identified and their different types of mechanism of action against various diseases suggested. Polyphenols are a structural class of natural organic chemicals characterized by the presence of large multiples of phenol units. The number and characteristics of these phenol substructures underlie the unique physical, chemical and biological properties of particular members of the class (Sharma *et al.*, 2005).

According to modern medicine, most of the diseases are caused by the over production of free radicals. These free radicals damage the biological macromolecules including oxidation of sulfhydryl containing enzymes,

modification of amino acids, loss of function and degradation of proteins, damage of polysaccharides and extensive DNA strand breaks (Kuraoka *et al.*, 2001). To protect the action of highly reactive oxygen species (ROS), the body has several safeguard mechanisms. They include enzymes such as superoxide dismutase, glutathione peroxidase, catalase, etc. Many antioxidants in the body such as vitamin E and ascorbic acid also inhibit the generation of oxygen free radicals. Sometimes these protective mechanisms are found not sufficient to the insult produced by excess stress. So, the increased level of ROS cannot be neutralized by enzymes, this will lead to the damaging of the cells and the condition is known as oxidative stress. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Nergard *et al.*, 2005; Velioglu *et al.*, 1998; Wong *et al.*, 2006). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing (Pokorny *et al.*, 2001). In these circumstances supplementation of non-toxic antioxidants may have a chemoprotective role in the body (Logani and Davis, 1979). Most of the plant related compounds also have the ability to activate free radical scavenging enzymes.

Inflammation is the body's basic response to injury, in which white blood cells and chemicals protect the body from infection and repair injury. The immune system sensing inflammation or irritation creates a protein chain called Circulating Immune Complex (CIC) which is specifically tagged to infection. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue

injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti *et al.*, 1995). Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di Rosa *et al.*, 1971) whereas prostaglandins are detectable in the late phase of inflammation (Pham-Marcou *et al.*, 2008).

Keratinization is the formation of keratin or development of horny epithelial layers. Keratin is a highly durable protein that provides structure to several types of living tissues. It is a major component of mammalian hair, nails and the outermost layer of skin and provides a tough, fibrous matrix to these tissues. An important quality of keratin is its ability to flex in multiple directions without tearing. However, excessive production of keratin in uterus at the endometrial epithelial cells hardens the uterus wall and leads to the breakage and bleeding, a condition called hemorhagea.

In rodents, responsiveness of genital tract epithelia to steroid hormones has been studied by several workers (Kumar *et al.*, 2002). Recently, attempts have been made to understand the mechanism of action of estradiol on vaginal epithelial cells (Urasopon *et al.*, 2008). Vaginal cells respond to estradiol and express the effects of the hormone even *in vitro*. The cells grow and terminally differentiate into keratinized cells. Studies have been conducted on the induction of keratinization of uterus by the administration of estrogen which is a steroid hormone in rats (Parhizkar *et al.,* 2011). On administration of estrogen, the original 3 cell layered

cuboidal epithelium of uterus changes to a stratified, multilayered, fully keratinized epithelium. The subsequent establishment of a keratin layer is preceded by the appearance of keratohyaline granules and the disappearance of mitochondria and endoplasmic reticulum in cells. (Vijayasaradhi *et al.*, 1987). A variety of systemically administered chemical agents interfere with the development of keratinized structures. These agents include vitamin A, thallium, antineoplastic compounds, hypocholesteremic drugs, chloroprene dimers, mimosine and anticoagulants.

Reports suggest that various preparations of *Saraca asoca* show antiestrogenic activity (Pradhan *et al.*, 2009). Ashokarishtam, a classical preparative medicine is widely used in the management of menorrhagia. Menorrhagia is an abnormally heavy and prolonged menstrual period at regular intervals due to disruption of normal hormonal regulation of periods, disorders of the endometrial lining of the uterus or abnormal blood clotting. The treatment with Ashokarishtam controls the excessive uterine bleeding, enhances the repair of the endometrium, eliminates fibroids, balanced production of female hormones for regular and healthy menses/periods of menstruation.

Studies have shown that *Saraca asoca* and its preparation asokarishtam inhibit cyclooxygenase (COX) activity which is the prime responsible enzyme in inflammatory processes (Deepti *et al.*, 2011). Cyclooxygenase (COX, also known as prostaglandin G/H synthase) is a membrane bound enzyme responsible for the oxidation of arachidonic acid to prostaglandin G2 (PGG2) and the subsequent reduction of PGG2 to prostaglandin H2 (PGH2). These reactions are the first steps in the formation of a variety of prostanoids. COX has been shown to be expressed in at least two different isoforms, a constitutively expressed form, COX-1, and an inducible form, COX-2. COX-1 is thought to regulate a number of 'housekeeping' functions, such as vascular hemostasis, renal blood flow,

and maintenance of glomerular function. Inflammation mediators such as growth factors, cytokines, and endotoxin induce COX-2 expression in a number of cellular systems (Klivenyi *et al.*, 2004). The effect of various non-steroidal anti-inflammatory drugs (NSAIDs) on the activity of COX-1 and -2 is an area of considerable interest.

The present study, funded by the National Medicinal Plants Board, New Delhi, was carried out to assess the phytochemical and biological properties of *Saraca asoca* and its substitutes, *Kingiodendron pinnatum, Cynometra travancorica*, *Humboldtia vahliana* and *H. brunonis* and *Kaempferia rotunda* and its substitutes *Lagenandra* species and Ayurvedic medicines such as 'Asokarishtam' and 'Drakshadikashayam' prepared with preferred species as well as substitutes with the following objectives.

- Qualitative and quantitative characterization of phytochemicals in *Saraca asoca* and *Kaempferia rotunda* and their substitutes
- Preparation of a comparative account of phytochemical profiles of *Saraca asoca* and *Kaempferia rotunda* and other related species.
- Evaluation of the general biological properties such as antioxidants, anti-inflammatory properties etc. of 'Asokarishtam' and 'Drakshadi Kashayam' prepared with preferred species as well as substitutes.
- The specific biological activities of 'Asokarishtam' prepared with preferred species as well as substitutes by measuring cornification of the uterus epithelium, the estrogenic level and testing the enzymes involved in prostaglandin synthetase.

2. MATERIALS AND METHODS

2.1. Profile of selected medicinal plants

Saraca asoca (Roxb.) de Wilde

Synonym: S.indica sensu Bedd. non L.

Ashokam (Mal.); Asoka tree (Eng.)

Erect, small, evergreen trees; bark 4-5 mm thick, dark brown, often warty, reddish-brown inside. Leaves alternate, up to 30 cm long, pinnate; leaflets 4-6 pairs, c. 10-20 cm long, oblong, lanceolate, glabrous. Flowers in dense corymbs, changing from yellow to orange and finally to scarlet; bracts and bracteoles coloured. Calyx situated at the top of tubular 12-17 mm long hypanthium. Sepals 4, ovate, petaloid. Petals absent. Stamens 4-8, exserted. Ovary stipitate, style curved into a ring, nearly as long as the stamens. Pods 15-25 cm long, dehiscent; seeds 2-8 (Plate 1a).

Habitat: Evergreen forests, also grown as ornamental tree in the plains

Kingiodendron pinnatum (Roxb. ex DC.) Harms

Chukennappayin, Churali, Ennappayin *(Mal.)*; Malabar mahogany *(Eng.)*

Large evergreen trees; bark 8-11 mm thick, smooth, brownish-grey, mottled with green, dull red inside. Leaves alternate, to 18 cm long, pinnate; leaflets 5-7, oblong-lanceolate, acuminate, base truncate, glabrous on both sides, to 11 x 4 cm; lateral nerves many, parallel; petiole 2-3 cm long; petiolule 0.5-1 cm. Flowers subsessile, white, 4 mm across in axillary and terminal paniculate spikes. Sepals broadly ovate. Petals 0. Stamens longer than the sepals. Pods ovate-ellipsoid, turgid, obtusely beaked, prominently veined, 4 x 2.5 cm; seed one (Plate 1b).

Habitat: Evergreen forests

Cynometra travancorica Bedd.

Koori *(Mal.)*

Evergreen trees, bole shortly buttressed, bark 3-4 mm thick, surface reddish-brown, smooth, lenticellate; reddish-brown inside. Leaves alternate, 2-foliate; leaflet 8-12 x 2-3 cm, often falcate, ovate-oblong, oblong or lanceolate, unequal sided; lateral nerves 6-8 pairs, pinnate, slender, prominent. Flowers 6-8 mm across, rosy white, in short dense flowered axillary racemes; bracts ovate, 1-3 mm, puberulous and ciliate; bracteole ovate, 2 m long. Sepals 4, 3 mm long, oblong, reflexed, glabrous. Petals 5, 4 mm long, subequal, clawed. Stamens 10, alternate long and short, connective cleft at base, very slightly apiculate. Ovary half inferior, semi-lunar, glabrous. Fruit a pod 2.5-3 cm long, flat, smooth, rugose along the margin, semicircular, the inner suture nearly straight, the outer one widely curved, indehiscent; seed one (Plate 1d).

Habitat: Semi-evergreen and evergreen forests

Cynometra beddomei Prain

Cherukoori *(Mal.)*

Evergreen trees, bark blackish-green; dull red inside. Leaves paripinnate, alternate; leaflets 4-6, opposite, 2.5-5 x 1-1.8 cm, obliquely obovate-oblong or obovate, base oblique, acute or cuneate, apex obtusely acuminate, emarginate; lateral nerves 6-8 pairs. Flowers creamy-white, in axillary clusters; peduncle 12-20 mm; bracts ovate, appressed, hairy, ciliate, striate, imbricate, at length deciduous, smaller upwards; pedicel 5 mm, pubescent. Sepals 3 mm long, hairy, ciliate. Petals 5, 3.5 x 1 mm, free, oblanceolate, subequal, glabrous. Stamens 10, alternately 5 and 7 mm long. Ovary half inferior, densely long brown hairy. Fruit a pod, reniform-globose, grooved near sutures, indehiscent; seed one (Plate 1e).

Habitat: Evergreen and semi-evergreen forests

Humboldtia brunonis Wall.

Kattasokam, Kaduasoka *(Mal.)*

Small evergreen trees, bark dark brown to black; pink-red inside; branchlets solid, sometimes swollen. Leaves paripinnate, alternate, stipulate; stipules 1-2 x 0.25-0.5 cm, lateral, linear, prominently parallel veined, glabrous, depressed glandular; appendages broadly reniform; rachis 20-40 mm long, obscurely winged; leaflets (2)-4, opposite; 7-20 x 2.2-8 cm, obovate oblong, oblong or elliptic-oblong, base unequal, apex obtusely acuminate; lateral nerves 7-13 pairs. Flowers 2-3 cm long, white, in axillary pendulous, pubescent, racemes; pedicels 4-5 mm long, pilose; bracts ovate, acute, pubescent; bracteoles 2, connate below. Calyx tube 8-10 mm long, lobes 4, concave, imbricate. Petals 3, white, pink or orange, ovate, shortly clawed. Stamens 5, perfect, free, alternating with 5 minute staminodes. Ovary 5 mm long, half inferior, obliquely linear, densely pubescent; ovules 3-5; style filiform, 10 mm long; stigma capitate. Fruit a pod 5-10 x 2-4 cm, dolabriform, brown pubescent when young; seeds 3-4 (Plate 1c).

Habitat: Evergreen forests

Humboldtia vahliana Wight

Aattuvanchi, Karappongu *(Mal.)*

Small to medium sized evergreen trees; bark rough, dark brown inside. Leaves alternate, to 30 cm long; leaflets 3 or 4 pairs, oblong – lanceolate or lanceolate, acuminate, base obtuse, glabrous, terminal leaflets to 25 x 5.5 cm; lateral nerves 7-9, thin; petiole 2-2.5 cm long; stipule foliaceous, ovate with reniform appendage at base, to 2.5 cm long. Flowers white, 2.5 cm across, in many flowered reflexed, to 15 cm long; racemes; bracts ovate acute, brown tomentose. Calyx tube 8-10 mm long, lined by the disc, obconical, brown villous; lobes 4, obovate-oblong, rounded at apex, slightly concave. Petals 5, 10-12 x 4 mm, obovate, shortly clawed, broadly rounded at apex, glabrous Pods

oblong, compressed, adpressed reddish brown tomentose, margins thickened, reticulate, to 15 x 4.5 cm; seeds 3-4, almost orbicular, glabrous (Plate 1f).

Habitat: Along river banks in semi-evergreen and evergreen forests

Kaempferia rotunda L.

Malan-kua, Chengazhi, Chengazhineerkizhangu *(Mal.)*

Herbs; rhizome short, stout, 2.5-3.5 x 2 cm strongly aromatic; roots stout, fleshy, often terminating in ovoid or spindle-shaped, 2-5 x 0.5 cm, yellow-white tubers. Leafy shoot to 50 cm high. Leaves few, radical, erect; lamina, 15-30 x 5-12 cm, oblong-lanceolate, with acute base and gradually acuminate apex, purple beneath, mottled green above, upper surface glabrous, lower surface densely covered with very short hairs; petiole 6-8 cm long. Inflorescence appearing before the leaves, shortly peduncled, enclosed within greenish-purple, narrow sheaths; 4-12 flowered. Calyx 5-6 cm long, light violetish, sparsely hairy. Corolla tube 6-7 cm long, slender, obliquely funnel-shaped towards the mouth; lobes white. Labellum 5.5-6 x 2-2.5 cm, broadly ovate, deeply divided into 2 suborbicular lobes, lilac with deep violet in the centre, with many radiating violet lines. Lateral staminodes 5-5.5 x 1.6 cm, ovate elliptic, tip acuminate, white with a violet tinge towards the margin. Filament short, erect, 5 mm long, upper half light violet. Ovary 5 x 3 mm, tricarpellary, with many ovules on axile placenta, glabrous (Plate 1g).

Habitat: Cultivated

Lagenandra ovata (L.) Thw.

Aandavazha, Karin-pola *(Mal.)*

Procumbent marshy herbs; rhizome 2-3 cm diameter, creeping. Leaves 10-25 x 5-12 cm, oblong to elliptic lanceolate, acute at apex, round to broadly attenuate at base, brownish black on drying; petioles

6-12 cm long, sheathing at base. Inflorescence axillary; peduncles short, stout. Spathe tubular below with margins united; limb long, acuminate, expanded in the middle; muricate with out. Spadix included with in the tube. Flowers unisexual, female at base, male at the top, both separated by a barren slender sterile interstice. Stamens 1-2. Ovary 1- celled; ovules 1-2 or many. Infructescence globose. Fruit a capsule, split opening from a base; seeds ribbed (Plate 1h).
Habitat: Marshy areas and stream sides

Lagenandra toxicaria Dalz.
Andavazha, Neerchengazhi *(Mal.)*

Procumbent or erect marshy herbs; rhizome to 4 cm diameter. Leaves 30-40 x 10-15 cm, oblong-acuminate; petiole cylindrical ca. 40-50 cm long. Inflorescence with a short peduncle of ca. 2 cm long. Spathe ca. 13-15 cm long, light greenish pink or pinkish cream in colour, purplish with darker vertical ridges inside. Spadix very small, ca. 1.8 cm long with basal pistillate portion followed by a slender barren interstice of ca. 3 mm long, a staminate portion, and terminating into a short barren appendix. Fruit a more or less fleshy capsule; dehiscence by 3-4 longitudinal splits from the base upwards at maturity; seeds ca. 1.6 mm long, ovoid-ellipsoid, slightly bent, longitudinally ridged (Plate 1i).
Habitat: Along streams

Collection of plant samples

Kingiodendron pinnatum, Cynometra travancorica and *Humboldtia brunonis* were collected from Wayanad dist. *Humboldtia vahliana, Lagenandra toxicaria* and *L. ovata* from Vazhachal, Thrissur dist. *Kaempferia rotunda* was collected from the medicinal plants garden, KFRI, Peechi, Thrissur.

a. *Saraca asoca*

b. *Kingiodendron pinnatum*

c. *Humboldtia brunonis*

d. *Cynometra travancorica*

e. *Cynometra beddomei*

f. *Humboldtia vahliana*

g. *Kaempferia rotunda*

h. *Lagenandra ovata*

i. *Lagenandra toxicaria*

PLATE 1

Extraction of metabolites

The samples were dried in hot air oven at 50º C and powdered using mixer grinder. The powder of each sample was successively extracted using soxhlet extraction system with various solvents of different polarity. The solvents of all extracts were evaporated and subjected to further analysis.

2.2. Phytochemical analysis

For phytochemical analysis, the plant extracts were subjected to Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) analyses.

2.2 a. Thin Layer Chromatography (TLC)

The solvent extracts of plants were separated through TLC with various solvent systems for the comparative analysis of the secondary metabolites like polyphenolics, flavonoids and terpenes between *Saraca asoca* and its substitutes and *Kaempferia rotunda* and its substitutes

Solvent system used for chromatographic separation

- Chloroform : acetic acid (9:1)
- Ethyl acetate : benzene (9:11)
- Benzene : ethyl acetate (19:1)
- Petroleum benzene : acetone : methanol (4:4:2)
- Acetone : methanol : water (4:3:3)
- Petroleum benzene : acetone : methanol : water (2:4:3:1)

2.2 b. High Performance Thin layer Chromatography (HPTLC)

Approximately 4 g dried powder of each plant sample was extracted with 40 ml methanol using soxhlet extraction system (Soxtec, Switzerland). Pre-coated 0.2 mm thick 20 x 20 cm size silica gel TLC plates were (E. Merck, Germany) used for the study. Five milligrams of extract was dissolved in 1.0 ml of methanol and subjected to ultra-sonication for ensuring proper mixing and dilution. The samples (5 μL) were spotted on

silica plate by Linomat 5 automatic sample spotter (Camag, Switzerland) and developed in glass twin trough chamber. The separating profile was docked and scanned by TLC scanner linked to winCATS software. A total of seven solvent systems such as chloroform:acetic acid (9:1); methanol:chloroform (1:1); toluene:ethylacetate: formic acid:methanol (3:6:1.6:0.4); hexane: ethylacetate:methanol (4:5:1); toluene:ethyl acetate (9.3:0.7); butanol:acetic acid:water (5:1:4) and butanol:acetic acid:water (5:1.3:4) were tried for the separation of compounds on the plates.

Among the solvent systems used, butanol: acetic acid: water (5:1.3:4) showed better separating profile for the loaded sample. The chromatogram developed was photographed and scanned at 254 and 366 nm before derivatization. After derivatization with anisaldihyde–sulphuric acid reagent (AS), the chromatogram was scanned at 580 nm. The densitometric graph of each substitute species was compared with that of original species

2.3. Biological analysis

The effect of plant extracts and Ayurvedic formulations prepared with *Saraca asoca* and its substitutes was biologically evaluated. Biological analyses included antioxidant and anti-inflammatory activities. Effect of plant extracts on estrogen induced keratinization and comparative antiestrogenic activity of *Saraca asoca* and substitutes were performed in rat uterus.

Animals

Female Swiss Albino mice (body weight 25-30 g) were purchased from Small Animal Breeding Station, College of Veterinary, Agricultural University, Thrissur, Kerala. The animals were maintained under standardized environmental conditions (22-28^{o} C, 60-70% relative humidity, 12 hr dark/light cycle) and fed with standard rat feed (Lipton, India) and water *ad libitum*. All animal experiments were carried out at

Amala Cancer Research Centre with the prior permission of Institutional Animal Ethics Committee (IAEC).

Preparation of extracts

The extracts isolated through soxhlet extraction system were filtered, concentrated and evaporated to dryness. The dried extracts were dissolved in phosphate buffer saline (PBS) and used for further biological studies.

2.3. a. *In vitro* antioxidant activities

The antioxidant properties of the plants were analysed by determining the scavenging effects of free radicals such as superoxide, hydroxyl radical and lipid peroxidation generated with *in vitro* assay systems.

Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined by the Nitroblue Tetrazolium (NBT) reduction method (McCord and Fridovich, 1969). Reaction mixture contained 6 μM ethylene diamine tetra acetic acid (EDTA), 0.0015% NaCN, 2μM riboflavin, 50 μM NBT, various concentrations of extract and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 minutes and the optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide radical generation was evaluated by comparing the absorbance values of control and experimental tubes.

Hydroxyl radical scavenging activity

Hydroxyl radicals generated from Fe^{2+}/ascorbate/H_2O_2 system degrades deoxyribose producing thiobarbituric acid reacting substance (TBARS) (Elizabeth and Rao, 1990). The efficacy of the extracts to inhibit TBARS formation was assessed. The reaction mixture

contained 2.8 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 1 mM H_2O_2, 0.1 mM ascorbic acid, 20 mM KH_2PO_4-KOH (pH 7.4) and various concentrations of extracts in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37° C. The TBARS formed was measured by the method of Ohkawa *et al* (1979) and the percentage inhibition was calculated from the optical measurements of control and experimental tubes.

Lipid peroxidation assay

The assay system contained 0.1 ml rat liver homogenate (25 % w/v) in 20 mM Tris-HCl buffer (pH 7.0), 30 mM KCl, 0.16 mM $FeSO_4$ $(NH_4)_2SO_46H_2O$ and 0.06 mM ascorbic acid and various concentrations of extract in a final volume of 0.5 ml and was incubated for 1 hr at 37° C. After incubation, 0.1 ml was removed and treated with 0.2 ml 8% sodium dodecyl sulphate (SDS), 1.5 ml 0.8% TBA and 1.5 ml 20% acetic acid (pH 3.5). The total volume was made up to 4 ml by adding distilled water and kept in water bath at 95° C for 1 hr. After cooling, 1 ml distilled water and 5 ml butanol-pyridine mixture (15:1, v/v) were added. Following vigorous shaking, the tubes were centrifuged at 3,000 rpm for 10 minutes. The organic layer was removed and its absorbance was measured at 532 nm. Inhibition of lipid peroxidation in the treated samples was determined by comparing the optical density to that of control.

DPPH radical scavenging assay

Stable radical, 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in methanol was used as substrate to evaluate anti-oxidant activity. The method is based on the reduction of DPPH radical in the presence of hydrogen donating antioxidants leading to the formation of a non-radical form DPPH-H by the reaction. DPPH in its radical form has an absorption

peak at 515 nm which disappeared upon the reduction by antioxidant compounds. Absorbance was measured 20 minutes after the reaction was started.

ABTS scavenging activity

ABTS (2, 2-azobis -3-ethylbenthiozoline-6-sulfonic acid) radical scavenging activity of the extract was determined by the method described by Alzoreky and Nakahara (2001). The principle involves the oxidation of ABTS to its cation radicals by ferryl myoglobin formed in the reaction of H_2O_2 and metmyoglobin. Briefly, the stock solutions of 500 μM ABTS diammonium salt, 400 μM myoglobin (MbIII), 740 μM potassium ferrycyanaide and 450 μM H_2O_2 were prepared in PBS (pH 7.4). Metmyglobin was prepared by mixing equal volumes of myoglobin and potassium ferrycyanide solutions. The reaction mixture (2 ml) contained ABTS (150 μM), MbIII (2.25 μM), varying concentrations of extracts and PBS. The reaction was initiated by adding 75 μM H_2O_2 and oxidation reaction was monitored at 734 nm using spectrophotometer.

Ferric reducing antioxidant power assay (FRAP)

The FRAP reagent contained 2.5 ml 2, 4, 6-Tris (2-pyridyl)-s-triazine (TPTZ), 20 mM 2.5 ml ferric chloride solution and 25 ml acetate buffer. Freshly prepared FRAP reagent (900 μl) was mixed with various concentrations of extracts and incubated at 37^{o} C for 15 min. The changes in absorbance at 595 nm were measured.

In all the experiments the percentage inhibition was calculated and concentration needed for 50% inhibition was found out by the formula as follows

$$\% \text{ inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

2.3 b. Anti-inflammatory activity

Acute and chronic anti-inflammatory assay systems were performed. To induce the acute and chronic inflammation on right hind paw carrageenan and formalin respectively were used.

Carrageenan induced paw edema

Animals were divided into different groups comprising 6 animals in each group. Acute inflammation was induced by sub-plantar injection of 0.02 ml freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of mice. One group with carrageenan alone served as positive control. The second group was administered with diclofenac (4 mg/kg body weight) intraperitoneally as standard reference drug. The other groups received extracts at the dosage of 50 and 100 mg/kg body weight, orally, 1 hour prior to the injection of carrageenan. The paw thickness was measured using vernier caliper before and 3 hours after carrageenan injection.

Formalin induced paw edema

Animals were divided into different groups comprising 6 animals in each group. Chronic inflammation was produced by injection of 0.02 ml of 1% formalin in the right hind paw of mice. One group was kept as control, the second group received standard reference drug diclofenac (4 mg/kg body weight) intraperitoneally, while other groups received 50 and 100 mg/kg body weight of extracts, orally one hour prior to formalin injection. The drug administration was continued for six consecutive days. The paw thickness was measured using vernier caliper before and 6 days after formalin injection.

Statistical analysis

The values are presented as mean ± SD. Difference between group means were estimated using a one way analysis of variance followed by Student's *t*-test. The results were considered statistically significant when $P < 0.05$.

2.3. c. Effect of plant extracts on estrogen induced keratinization

Animals were divided into six groups comprising six animals in each group. First group was kept as control (without any treatment), second group received vehicle control propanediol (0.05 ml), third group received estradiol, fourth, fifth and sixth group received 100 mg/kg body weight methanolic extracts of *Cynometra travancorica, Saraca asoca* and *Kingiodendron pinnatum*, respectively. The drug was administered orally for 5 days starting from 7th day after birth. Third, fourth, fifth and sixth groups animals were injected 20 μg estradiol in 0.05 ml of propanediol for 5 days starting from 7th day after birth (Plate 2). Animals were sacrificed at 8, 16, 24, 32, and 40 days after birth. The dissected uterus was kept in formalin. Sections of uterus were prepared and stained using hematoxylene-eosin stain. The histopathological analysis and the pattern of keratinization on endometrial of uterus was analysed microscopically using Leica Application Suit software (Plate 2).

2.3.d. Comparative antiestrogenic activity of *Saraca asoca* and substitutes

Animals were divided into four groups comprising 6 animals in each group. First group was kept as control (without any treatment), second, third, fourth group received 500 mg/kg body weight methanolic extract of *Saraca asoca, Kingiodendron pinnatum* and *Cynometra travancorica*, respectively. The drug was administered orally for 10 days on alternate days. On 10th day, the animals were sacrificed, serum collected and estrogen level was analyzed by

radioimmuno assay (RIA). Along with estrogen assay, the anticoagulated blood is used for measuring hemoglobin level and total lymphocyte (TC) and differential count (DC).

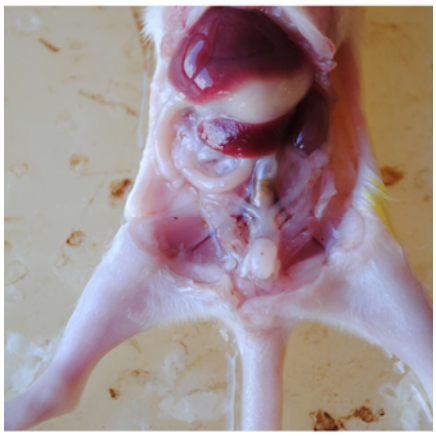
2.4. Preparation of Arishtams

Arishtams were prepared with *Saraca asoca* and its substitutes namely *Kingiodendron pinnatum*, *Cynometra travancorica*, *Humboldtia brunonis* and *Polyalthia longifolia* as per Indian Ayurvedic Pharmacopoeia. The stem bark was used for the preparation. Freshly collected barks were shade dried and chopped into small pieces. For preparing 1 litre arishtam, 4 litre water was boiled to which chopped bark was added. Boiling was continued until the volume got reduced to a quarter. Thereafter, sediments were removed by filtering. After adding sugar candy, the filtrate was boiled again for proper mixing. The filtrate (Kashayam) was transferred to a china clay jar and kept overnight. On the next day, chopped *Woodfordia fruticosa* (should not be powdered) flowers were added and mixed well. The jar was covered in a sequential manner such as:

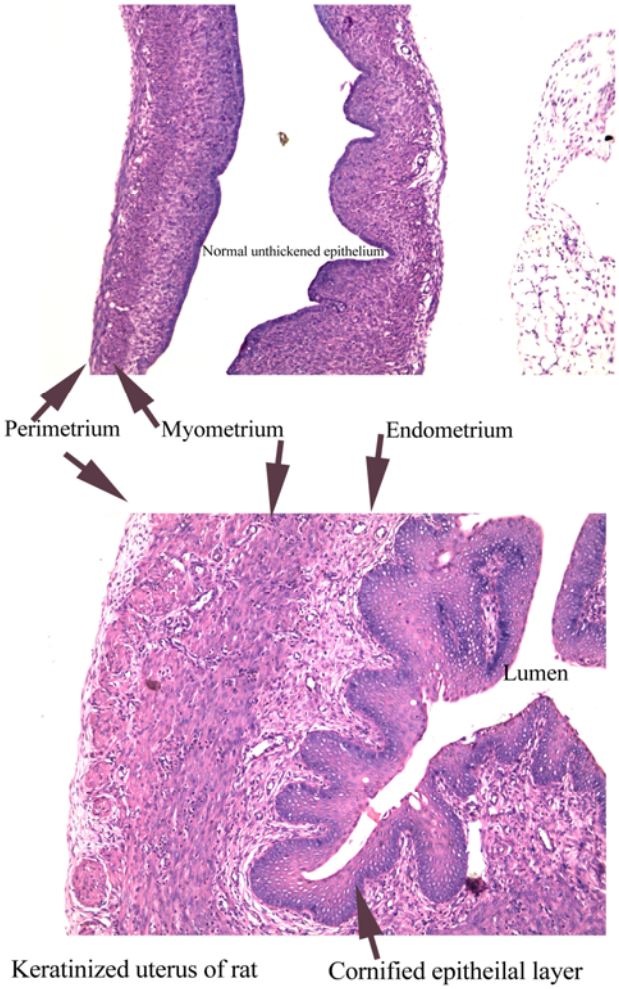
1. Place the lid with cloth.
2. Cover with cloth
3. Cover with polyethylene cover
4. Tie with twine
5. Place the cloth
6. Cover with paper
7. Tie with twine
8. Cover with cloth and seal with clay.

The jars were kept in dry area for 30 days. After 30 days, the Arishtam was filtered and is ready for use.

Female Wistar rat 7 days old



Dissected rat showing the uterus



Histopathology of rat uterus

PLATE 2

The ingredients to be used for preparing one litre of Arishtam are as follows:

Sl. No	Name	Parts Used	Quantity
01	*Saraca asoca*	Stem Bark	156.25 g
02	*Woodfordia fruticosa*	Dry flower	25 g
03	*Phyllanthus emblica*	Dry fruit	1.5625 g
04	*Coscinium fenestratum*	Stem	1.5625 g
05	*Cyperus rotundus*	Tuberous root	1.5625 g
06	*Justicia adhatoda*	Root	1.5625 g
07	*Terminalia bellirica*	Dry fruit	1.5625 g
08	*Terminalia chebula*	Dry fruit	1.5625 g
09	*Trachyspermum roxburghianum*	Seed	1.5625 g
10	*Cuminum cyminum*	Seed	1.5625 g
11	*Santalum album*	Heart wood	1.5625 g
12	*Kaempferia rotunda*	Rhizome	1.5625 g
13	*Mangifera indica*	Seed kernel	1.5625 g
14	*Zingiber officinale*	Dried rhizome	1.5625 g

In addition to these, 312 g sugar-candy and 4 litre water are required. In the same way Arishtams with *Kingiodendron pinnatum*, *Cynometra travancori ca*, *Humboldtia brunonis* and *Polyalthia longifolia* were prepared.

2.5. In vivo anti-oxidant analysis

Anti-oxidant activity of the Arishtams prepared with *Saraca asoca*, *Kingiodendron pinnatum* and *Cynometra travancorica* was evaluated using Swiss albino mice. Animals were divided in to 7 groups, each with 6 animals. First group was kept as control (without any treatment) and other groups received two doses, 200 and 400 µl/animal of each Arishtam. After 30 days of treatment, animals were

sacrificed. The blood collected and liver excised. Liver was washed in ice cold Tris HCl (0.1 M, pH 7.4). About 25% liver homogenate was prepared in 0.1M Tris HCl buffer and cytosolic samples of liver homogenate were prepared by centrifuging at 10,000 rpm for 30 minutes at 4^{o} C, to estimate the antioxidant enzymes. Superoxide dismutase activity was measured by the nitroblue tetrazolium (NBT) reduction method of McCord and Fridovich (1969). Catalase activity was estimated by measuring the rate of decomposition of hydrogen peroxide at 240 nm (Aebi, 1997). Glutathione (GSH) levels were assayed by the method of Moron *et al.* (1979) based on the reaction with 5,5-dithiobis 2-nitrobenzoic acid (DTNB). The assay of glutathione peroxidase was done by the method of Hafeman *et al* (1974) based on the oxidation of GSH forms in the presence of H_2O_2. The haematological parameters were also assessed.

2.5.a. Effect of Ayurvedic formulations (Arishtams) on estrogen induced keratinization

Animals were divided into eight groups comprising six animals in each group. First group was kept as control (without any treatment), the second group received vehicle control propanediol (0.05 ml), third group received estradiol, fourth, fifth, sixth, seventh and eighth groups received Arishtams prepared with *Saraca asoca*, *Kingiodendron pinnatum*, *Cynometra travancorica*, *Humboldtia brunonis* and *Polyalthia longifolia* respectively. The drug was administered orally for 5 days starting from 7th day after birth. Animals in the third, fourth, fifth, sixth, seventh and eighth groups were injected 20 µg estradiol in 0.05 ml of propanediol for 5 days starting from 7th day after birth. Animals were sacrificed at 8, 16, 24, 32, and 40 days after birth. The dissected uterus was kept in formalin. Sections of uterus were prepared and stained using hematoxylene-eosin stain. The histopathological analysis and the pattern of keratinization on endometrium of uterus was analysed microscopically using Leica Application Suit software.

2.5.b. Determination of cyclooxygenase (COX) activity by Western blot analysis on Arishtam treated mice

Wistar female rats, seven days old, were used for the study. The Arishtam was administered orally for 5 days starting from 7th day after birth. The animals were divided into 5 groups, group I kept as normal and group II was treated with lipopolysaccharide (LPS). Group III was treated with Asokarishtam orally for five consecutive days. Fourth and fifth groups received Arishtams prepared with *Kingiodendron pinnatum*, *Cynometra travancorica*, respectively. On the fifth day, group II and III animals were administered with LPS. After 90 minutes of administration of LPS, all groups of animals were sacrificed, the uterus collected and homogenized. Protein content was determined by Lowry's method. Protein extracts (20 μg) were heated at 95^{o} C for 5 min, resolved in 10% SDS-polyacrylamide gel. The separated protein bands were electroblotted to PVDF membrane using CAPS buffer for overnight at 30 mA. Membrane was then blocked in blocking buffer for 1 hour and washed with washing buffer for 5 min, three times each. Membrane was incubated with anti-COX-2 antibody (1:5000) for 1 hour (Alpha Diagnostics, USA) washed and incubated with Horse Radish Peroxidase (HRP)-conjugated anti-rabbit secondary antibody (1:5000) for 1 hour and the membrane developed using 3-3-diaminobenzidine (DAB)-substrate (Banglore GeNei).

3. RESULTS

3.1. Phytochemical analysis

3.1. A. Thin layer chromatography

3.1. A. a. Comparison between *Saraca asoca* and its substitutes

Comparison of polyphenolics

The acetone and methanolic extracts of stem bark of *Saraca asoca* and its substitutes were subjected to thin layer chromatography. The plates were sprayed with Folin-Ciocalteu's phenol reagent for phenolic compounds. Among the solvent systems used for thin layer cromatographphy, chloroform: acetic acid (9:1) and ethyl acetate: benzene (9:11) were found to give good separation profile. By spraying, 7 bands were observed for polyphenolic compounds with acetone extracts using both the solvent systems, chloroform: acetic acid (9:1) (Table 1) and ethyl acetate: benzene (9:11) (Table 2). The results show more or less similar patterns for polyphenolic compounds for *Saraca asoca* and its substitutes (Figures 1 and 2). However, the band with Rf. 0.26 was absent in *Saraca asoca* and *Kingiodendron pinnatum* and band with Rf. 0.42 was present only in *Humboldtia brunonis* in the profile obtained with the solvent system chloroform: acetic acid (9:1). In this profile, the substitute plant, *Kingiodendron pinnatum* showed similar banding pattern as that of *Saraca asoca* for polyphenolics compounds.

Comparison of flavonoids

The acetone extracts of stem bark were subjected to thin layer chromatography with various solvent systems and chromatogram was developed by spraying with Vanillin-HCl (Figure 3). While comparing *Saraca asoca* and its substitutes, only two bands were obtained by using

ethyl acetate: benzene (9:11) Result shows more or less similar patterns in all the samples (Table 3). In total, six bands were obtained when chloroform: acetic acid (9:1) was used as solvent system and sprayed with 10% aqueous lead acetate (Table 4).

Comparison of terpenoids

Acetone extracts of stem bark were subjected to thin layer chromatography. In total, 8 bands were obtained for *Saraca asoca* when ethyl acetate: benzene (9:11) was used as solvent system and 0.2% aqueous potassium permanganate as spray reagent (Figure 4). The result shows less similar patterns between the samples (Table 5). The band with Rf. 0.44 was observed only in *Humboldtia vahliana* and Rf 0.28 was found only in *Saraca asoca*. A total of 5 bands were obtained when chloroform: acetic acid (9:1) was used as solvent system (Figure 5 and Table 6). Result shows more or less similar patterns, but the band with Rf 0.45 was observed in *Humboldtia vahliana* and *Kingiodendron pinnatum*.

Table 1. Separation profile of phenolic compounds of *Saraca asoca* and its substitutes on TLC with chloroform: acetic acid (9:1) solvent system. The plate sprayed with Folin-Ciocalteu's phenol reagent

Sl. No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.08	+	+	+	+	+
2	0.26	-	+	+	+	-
3	0.42	-	-	+	-	-
4	0.47	+	+	+	+	+
5	0.63	+	+	+	+	+
6	0.75	-	+	-	+	-
7	0.96	+	+	+	+	+

Table 2. Separation profile of phenolic compounds of *Saraca asoca* and its substitutes on TLC with ethyl acetate: benzene (9:11) solvent system. The plate sprayed with Folin-Ciocalteu's phenol reagent

Sl. No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.22	+	+	+	+	+
2	0.34	+	-	-	-	-
3	0.50	+	+	+	-	+
4	0.56	+	+	+	+	+
5	0.72	+	+	-	-	+
6	0.90	+	+	+	+	+
7	0.96	+	+	+	+	+

Table 3. Separation profile of flavonoids of *Saraca asoca* and its substitutes on TLC with ethyl acetate: benzene (9:11) solvent system. The plate sprayed with Vanillin-HCl

Sl. No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.10	+	-	+	-	+
2	0.22	+	+	+	+	+

Table 4. Separation profile of flavonoids of *Saraca asoca* and its substitutes on TLC with chloroform: acetic acid (9:1) solvent system. The plate sprayed with 10% aqueous lead acetate solution

Sl. No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.02	+	+	+	+	+
2	0.20	-	-	+	+	+
3	0.45	-	-	-	+	-
4	0.54	-	-	-	+	+
5	0.85	-	-	-	+	-
6	0.9 7	+	+	+	+	+

Table 5. Separation profile of terpenoids compounds of *Saraca asoca* and its substitutes on TLC with ethyl acetate: benzene (9:11) solvent system. The plate sprayed with 0.2% aqueous potassium permanganate

Sl.No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.02	+	+	+	+	+
2	0.20	-	-	+	+	+
3	0.28	+	-	-	-	-
4	0.44	-	-	+	-	-
5	0.53	+	+	+	+	+
7	0.72	+	+	-	-	-
8	0.9 7	+	+	+	+	+

Table 6. Separation profile of terpenoids compounds of *Saraca asoca* and its substitutes on TLC with Chloroform: acetic acid (9:1) solvent system. The plate sprayed with 0.2% aqueous potassium permanganate

Sl.No.	Rf. Value	*S. asoca*	*C. travancorica*	*H. brunonis*	*H. vahliana*	*K. pinnatum*
1	0.02	+	+	+	+	+
2	0.20	+	+	-	-	+
3	0.45	-	-	-	+	+
4	0.65	+	+	+	+	+
5	0.94	+	+	+	+	+

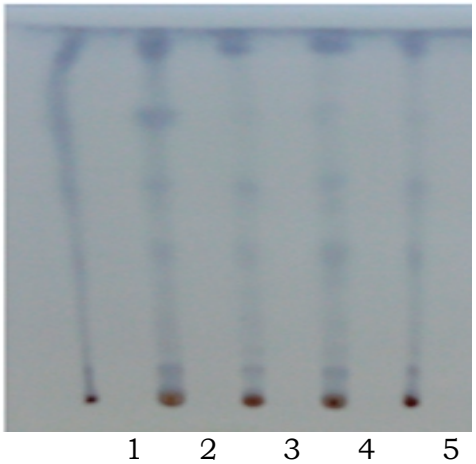
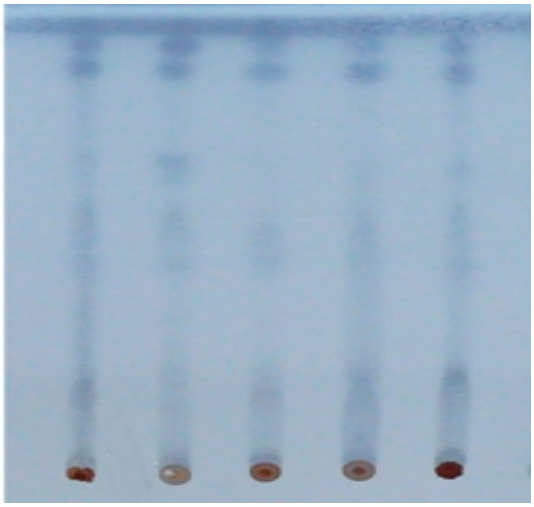
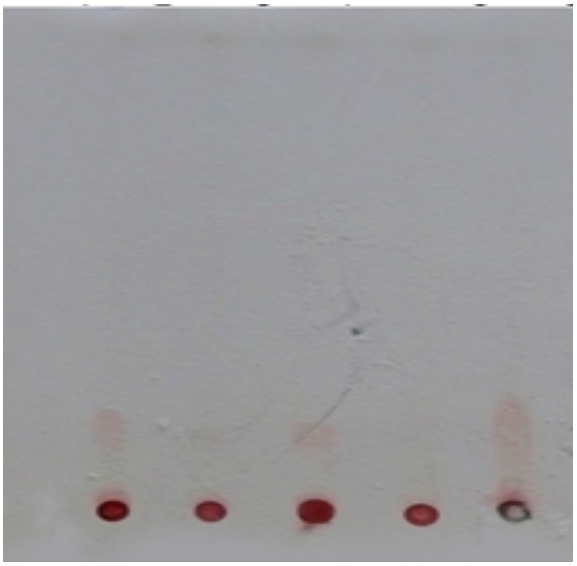


Figure 1. TLC analysis of methanolic extracts of *Saraca asoca* and substitutes with the solvent system, chloroform: acetic acid (9:1). Plate sprayed with spray reagent Folin-Ciocalteu's phenol reagent. Lane 1. *Saraca asoca*, 2. *Cynometra travancorica*, 3. *Humboldtia brunonis*, 4. *Humboldtia vahliana* and 5. *Kingiodendron pinnatum*



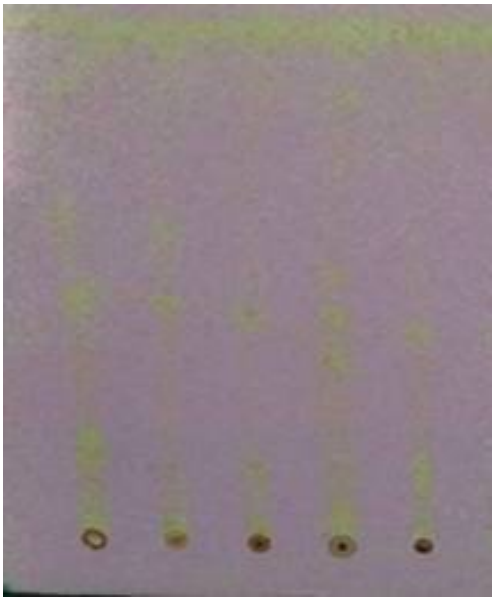
1 2 3 4 5

Figure 2. TLC analysis of acetone extracts of *Saraca asoca* and substitutes with the solvent system, ethyl acetate: benzene (9:11) and the plate sprayed with spray reagent Folin-Ciocalteu's phenol reagent. Lane 1. *Saraca asoca*, 2. *Cynometra travancorica*, 3. *Humboldtia brunonis*, 4. *Humboldtia vahliana* and 5. *Kingiodendron pinnatum.*



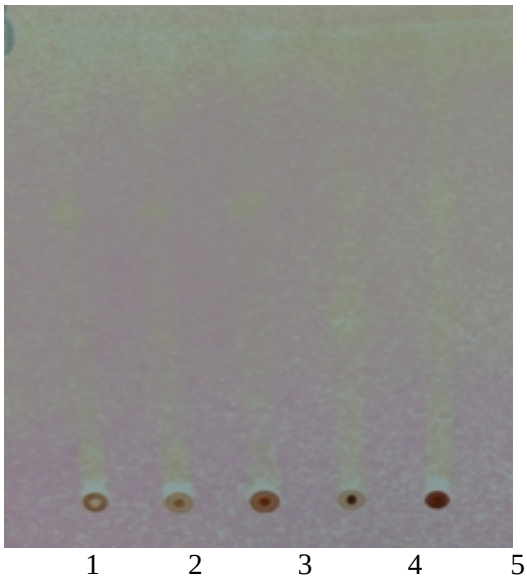
1 2 3 4 5

Figure 3. TLC analysis of acetone extracts of *Saraca asoca* and substitutes with the solvent system, ethyl acetate: benzene (9:11). Plate was sprayed with spray reagent Vanillin-Hcl. Lane 1. *Saraca asoca*, 2. *Cynometra travancorica*, 3. *Humboldtia brunonis*, 4. *Humboldtia vahliana* and 5. *Kingiodendron pinnatum*



1 2 3 4 5

Figure 4. TLC analysis of acetone extracts of *Saraca asoca* and substitutes with the solvent system, ethyl acetate: benzene (9:11). Plate rayed with spray reagent 0.2% aqueous potassium permanganate Lane 1. *Saraca asoca*, 2. *Cynometra travancorica*, 3. *Humboldtia brunonis*, 4. *Humboldtia vahliana* and 5. *Kingiodendron pinnatum*



1 2 3 4 5

Figure 5. TLC analysis of acetone extracts of *Saraca asoca* and substitutes with the solvent system, chloroform: acetic acid (9:1). Plate sprayed with spray reagent 0.2% aqueous potassium permanganate. Lane 1. *Saraca asoca*, 2.*Cynometra travancorica*, 3. *Humboldtia brunonis*, 4. *Humboldtia vahliana* and 5. *Kingiodendron pinnatum*

3.1. A. b. Comparison among *Kaempferia rotunda* and its substitutes

Comparison of polyphenolics

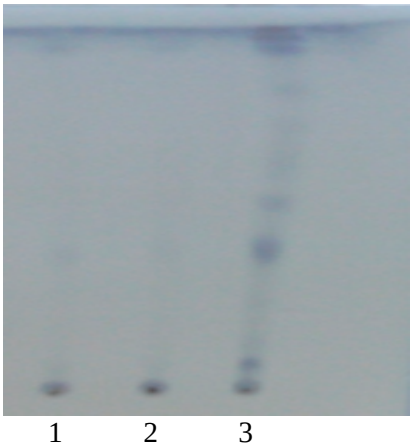
The acetone and methanolic extracts of rhizome of *Kaempferia rotunda* and its substitutes were subjected to thin layer chromatography. The plates were sprayed with Folin-Ciocalteu's phenol reagent for phenolic compounds. By spraying, *Kaempferia rotunda* produced 12 and 7 bands for polyphenolic compounds with acetone extracts using solvent system, chloroform: acetic acid (9:1) and ethyl acetate: benzene (9:11), respectively. Only 3 and 2 bands were separated by *Lagenandra toxicaria* and *Lagenandra ovata*, respectively for polyphenolic compounds with both the solvent systems (Figures 6 and 7).

Comparison of flavonoids

Clear banding pattern was not produced (Data not shown).

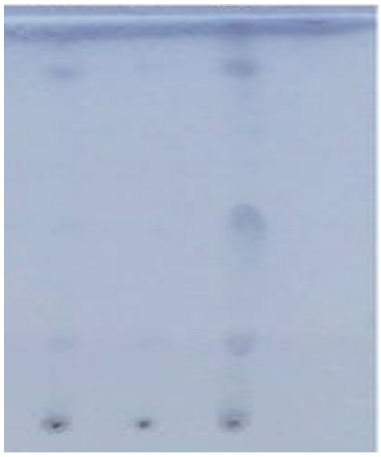
Comparison of terpenoids

Acetone extracts of stem were subjected to Thin Layer Chromatography. Altogether, 3 bands were obtained for *Lagenandra toxicaria* (Figure 8) with solvent system, ethyl acetate: benzene (9:11). For *Lagenandra ovata* and *Kaempferia rotunda* no clear banding pattern was observed.



1 2 3

Figure 6. TLC analysis of acetone extract of *Kaempferia rotunda* and substitutes with the solvent system, chloroform: acetic acid (9:1). Plate sprayed with spray reagent Folin-Ciocalteu's phenol reagent. Lane 1. *Lagenandra ovata*, 2. *Lagenandra toxicaria*, and 3. *Kaempferia rotunda*



1 2 3

Figure 7. TLC analysis of acetone extract of *Kaempferia rotunda* and substitutes with the solvent system, ethyl acetate:benzene (9:11). Plate sprayed with spray reagent Folin-Ciocalteu's phenol reagent. Lane 1. *Lagenandra ovata*, 2. *Lagenandra toxicaria* and 3. *Kaempferia rotunda*

1 2 3

Figure 8. TLC analysis of acetone extract of *Kaempferia rotunda* and substitutes with the solvent system, ethyl acetate: benzene (9:11). Plate sprayed with spray reagent 0.2% aqueous potassium permanganate. Lane 1. *Lagenandra ovata*, 2. *Lagenandra toxicaria* and 3. *Kaempferia rotunda*

The extracts obtained during soxhlet extraction were very low in quantity in the case of *Kaempferia rotunda* and substitutes *Lagenandra ovata* and *Lagenandra toxicariai*. Hence, the detailed studies were not performed in the case of *Kaempferia rotunda* and its substitutes.

3.1. B. High Performance Thin Layer Chromatography (HPTLC)

Scanning at 254 nm

A total of 8 bands were observed for *Saraca asoca* and *Kingiodendron pinnatum*. Five bands were observed for *Cynometra travancorica;* 8 for *Cynometra beddomei;* 7 for *Humboldtia brunonis* and 5 for *Humboldtia vahliana* (Table 7; Figures 9 and 10).

Table 7. Comparison of Rf bands at 254 nm

Sl.No	Rf	*S. asoca*	*K. pinnatum*	*C. travancorica*	*C. beddomei*	*H. brunonis*	*H. vahliana*
1	0.02	+	+	+	+	+	+
2	0.14	-	-	-	-	-	+
3	0.30	+	+	+	+	+	+
4	0.35	-	-	-	-	-	+
5	0.45	+	-	-	-	+	-
6	0.48	-	-	+	+	-	+
7	0.50	+	+	-	-	-	-
8	0.56	-	+	-	-	-	-
9	0.61	+	+	+	+	+	-
10	0.68	+	+	-	+	+	-
11	0.73	+	+	-	+	+	-
12	0.83	+	+	+	+	+	-
13	0.90	-	-	-	+	-	-

Scanning at 366 nm

A total of 10 bands were obtained for *Saraca asoca*; 13 for *Kingiodendron pinnatum;* 10 for *Cynometra travancorica;* 9 for *Cynometra beddomei;* 8 for *Humboldtia brunonis* and 10 for *Humboldtia vahliana* (Table 8; Figures 9B, 9C, 9D and 11).

Table 8. Comparison of Rf bands at 366 nm

Sl. No	Rf	*S. asoca*	*K. pinnatum*	*C. travancorica*	*C. beddomei*	*H. brunonis*	*H. vahliana*
1	0.01	+	+	+	+	+	+
2	0.20	+	+	+	+	+	+
3	0.24	-	-	-	-	-	+
4	0.30	+	+	+	+	+	-
5	0.35	-	-	-	-	-	+
6	0.36	+	+	+	+	+	-
7	0.41	-	+	-	-	-	-
8	0.44	+	+	-	-	-	-
9	0.46	-	+	+	+	+	+
10	0.48	+	+	-	-	-	-
11	0.57	-	+	+	-	-	+
12	0.60	+	+	+	+	-	-
13	0.66	+	+	+	+	+	-
14	0.70	-	-	+	+	-	+
15	0.73	+	+	-	-	+	-
16	0.79	-	-	-	-	-	+
17	0.82	+	+	-	+	+	+
18	0.89	-	-	-	-	-	+
19	0.96	-	-	+	-	-	-

Scanning at 580 nm

A total of 10 bands were found in *Saraca asoca*; 14 in *Kingiodendron pinnatum;* 8 in *Cynometra travancorica;* 8 in *Cynometra beddomei;* 11 in *Humboldtia brunonis* and 9 bands in *Humboldtia vahliana.*

Scanning after derivatization is more relevant for the comparative phytochemical profiling. Hence, we compared *Saraca asoca* with its substitutes with respect to its densitometric scan at 580 nm (Figures 9E, 12 and 13) with its scoring table (Table 9).

Table 9. Comparison of Rf bands at 580 nm

Sl. No	Rf	*S. asoca*	*K. pinnatum*	*C. travancorica*	*C. beddomei*	*H. brunonis*	*H. vahliana*
1	0.01	-	-	+	+	+	+
2	0.02	+	+	-	-	-	-
3	0.20	+	+	+	-	+	+
4	0.23	-	+	-	-	-	+
5	0.30	+	+	+	-	+	-
6	0.36	+	-	-	-	+	-
7	0.43	+	+	-	-	-	-
8	0.45	-	+	+	-	-	+
9	0.48	+	+	-	-	-	-
10	0.55	-	+	-	-	-	-
11	0.59	+	+	-	-	+	+
12	0.66	+	+	-	-	-	-
13	0.71	+	+	+	+	+	+
14	0.73	-	+	-	-	+	-
15	0.82	+	-	-	+	-	+
16	0.96	-	-	+	-	-	-

The band with Rf 0.20 was present in all samples except *Cynometra beddomei*. The band with 0.30 Rf was present in all samples except *Cynometra beddomei* and *Humboldtia vahliana*. The band with Rf. 0.36 was present only in *Saraca asoca* and *Humboldtia brunonis*. The bands 0.43, 0.48 and 0.66 Rf. were present in *Saraca asoca* and *Kingiodendron pinnatum* only. The band with 0.71 Rf was present in all the samples, except in *Kingiodendron pinnatum* and *Humboldtia brunonis*. The band with 0.82 Rf. was present in *Saraca asoca*, *Cynometra beddomei* and *Humboldtia vahliana*.

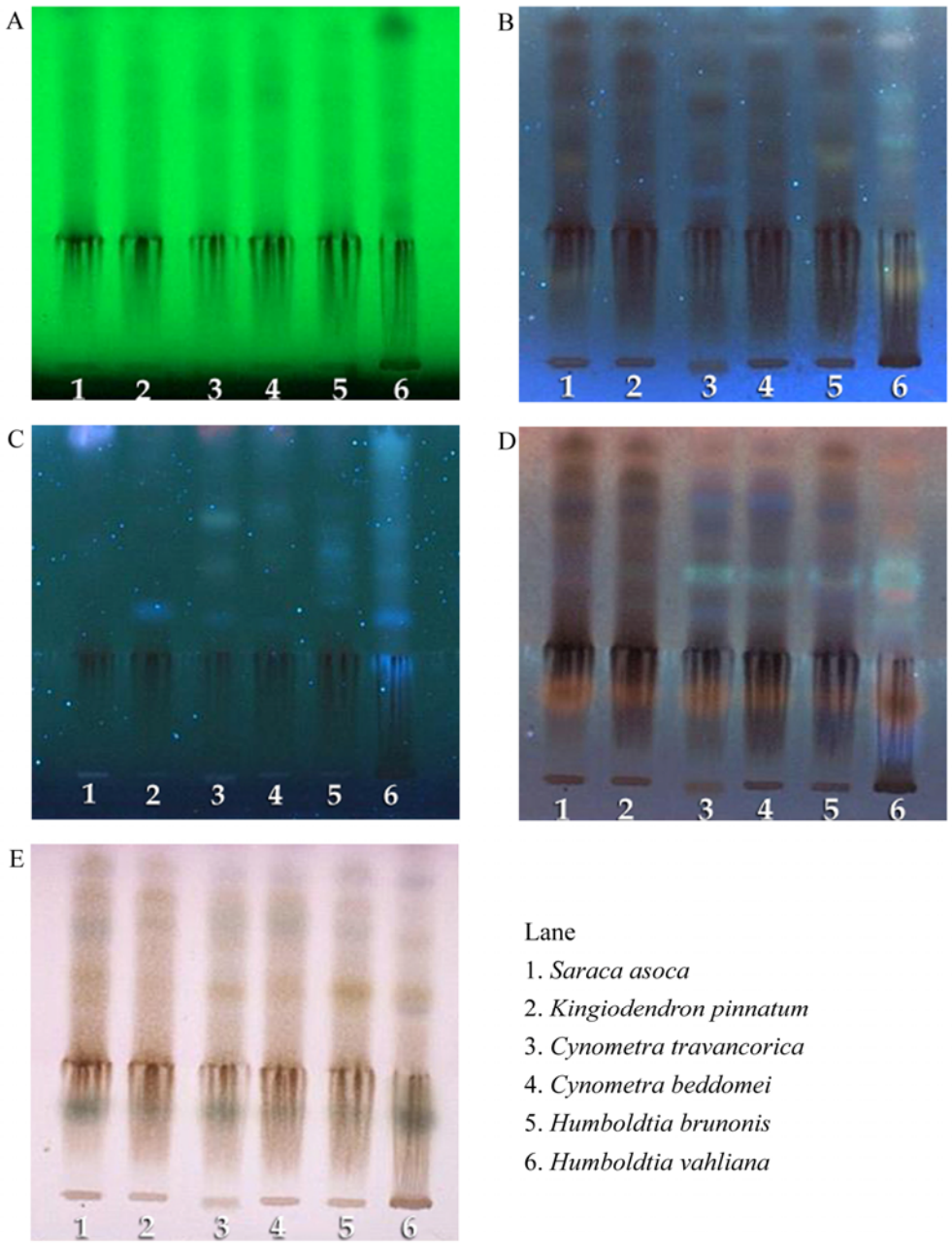


Figure 9. TLC photographs of plates developed in CAMAG glass twin trough chamber at different nanometer. Solvent system used was Butanol : Acetic acid : Water (5: 1.3:4) and sprayed with Anisaldihyde-Sulphuric acid (AS). (A) at 254 nm before derivatization (B) at 366 nm after derivatization (C) at 366 nm before derivatization (D) at 366 nm after derivatization of second heating and (E) at white light after derivatization.

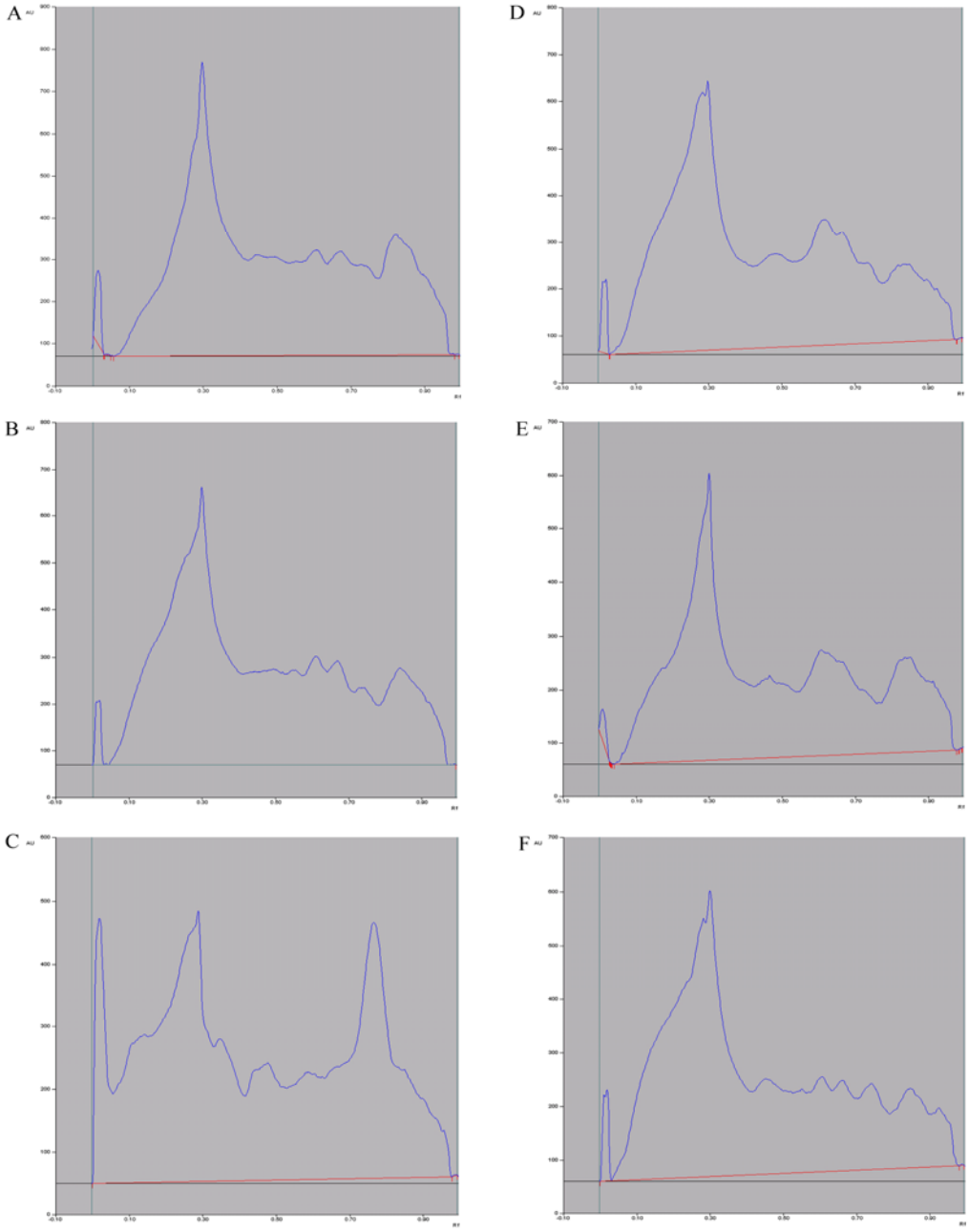


Figure 10. TLC Dencitometric scan at 254nm. A. *Saraca asoca*, B. *Kingiodendron pinnatum* C. *Humboldtia vahliana*, D. *Cynometra beddomei*, E. *Cynometra travancorica*, F. *Humboldtia brunonis*

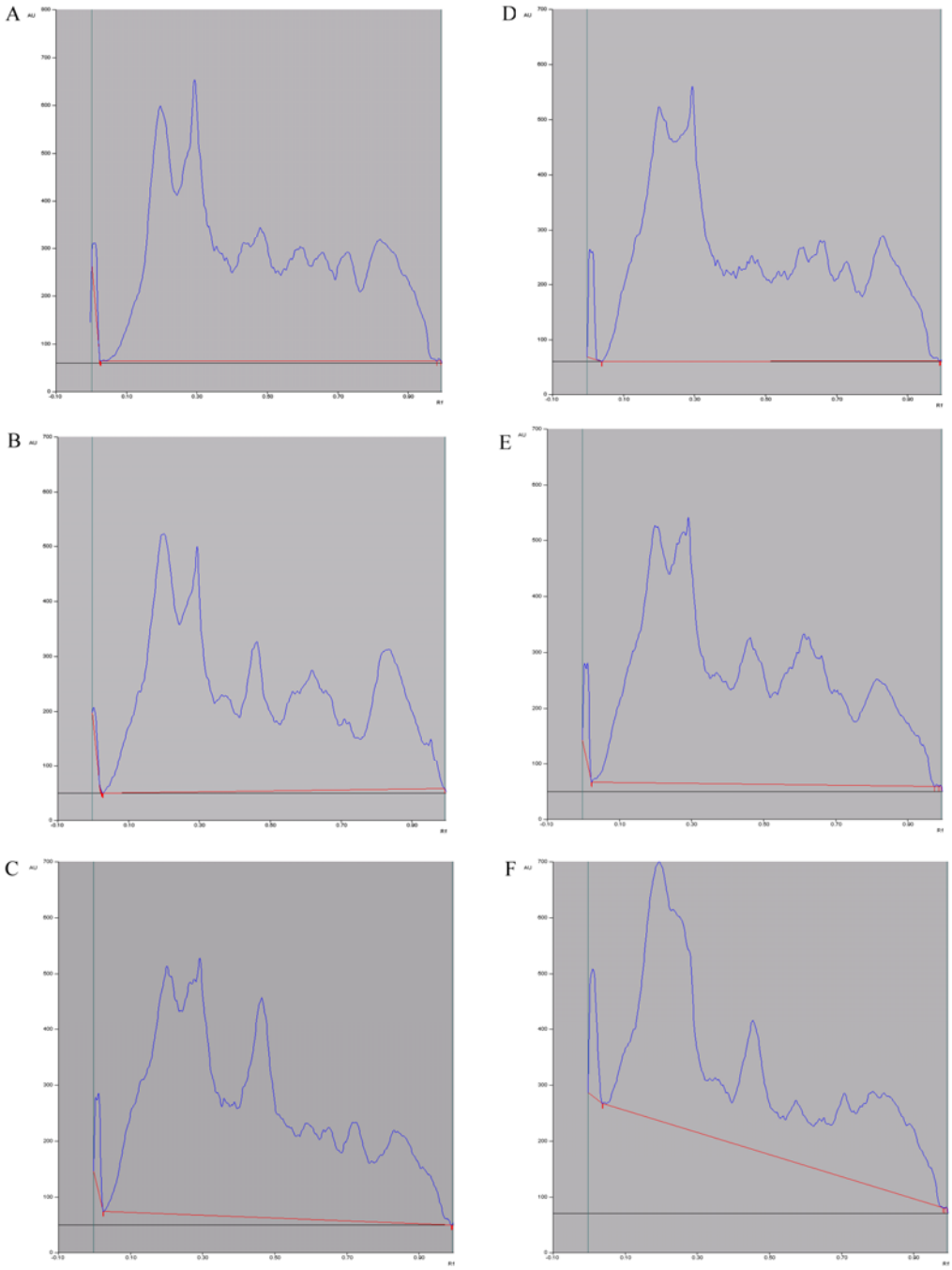


Figure 11. TLC Dencitometric scan at 366nm. A. *Saraca asoca*, B. *Cynometra travancorica*, C. *Humboldtia brunonis*, D. *Kingiodendron pinnatum*, E. *Cynometra beddomei*, F. *Humboldtia vahliana*

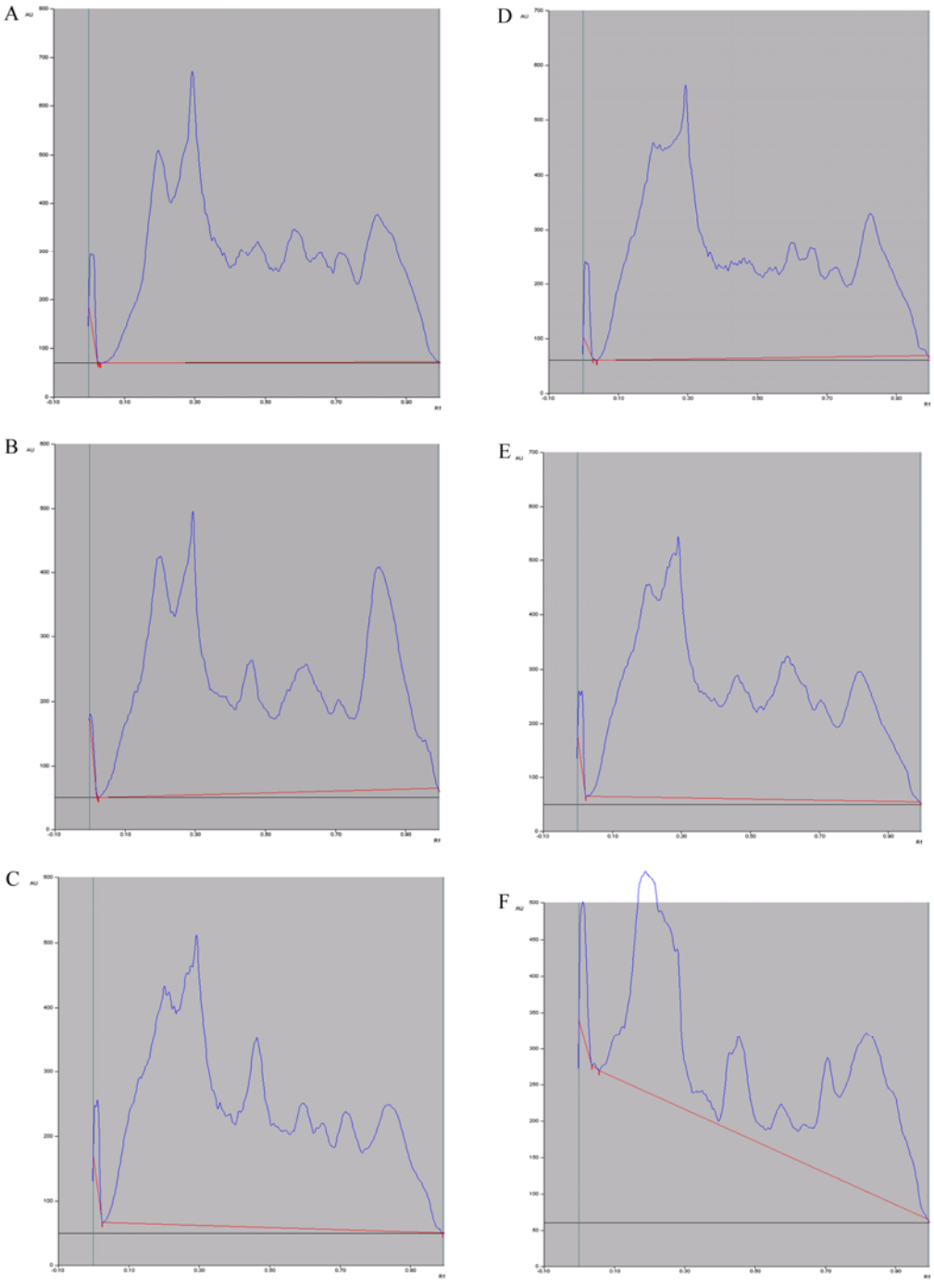


Figure 12. TLC Dencitometric scan at 580 nm. A. *Saraca asoca*, B. *Cynometra travancorica*, C. *Humboldtia brunonis*, D. *Kingiodendron pinnatum*, E. *Cynometra beddomei*, F. *Humboldtia vahliana*

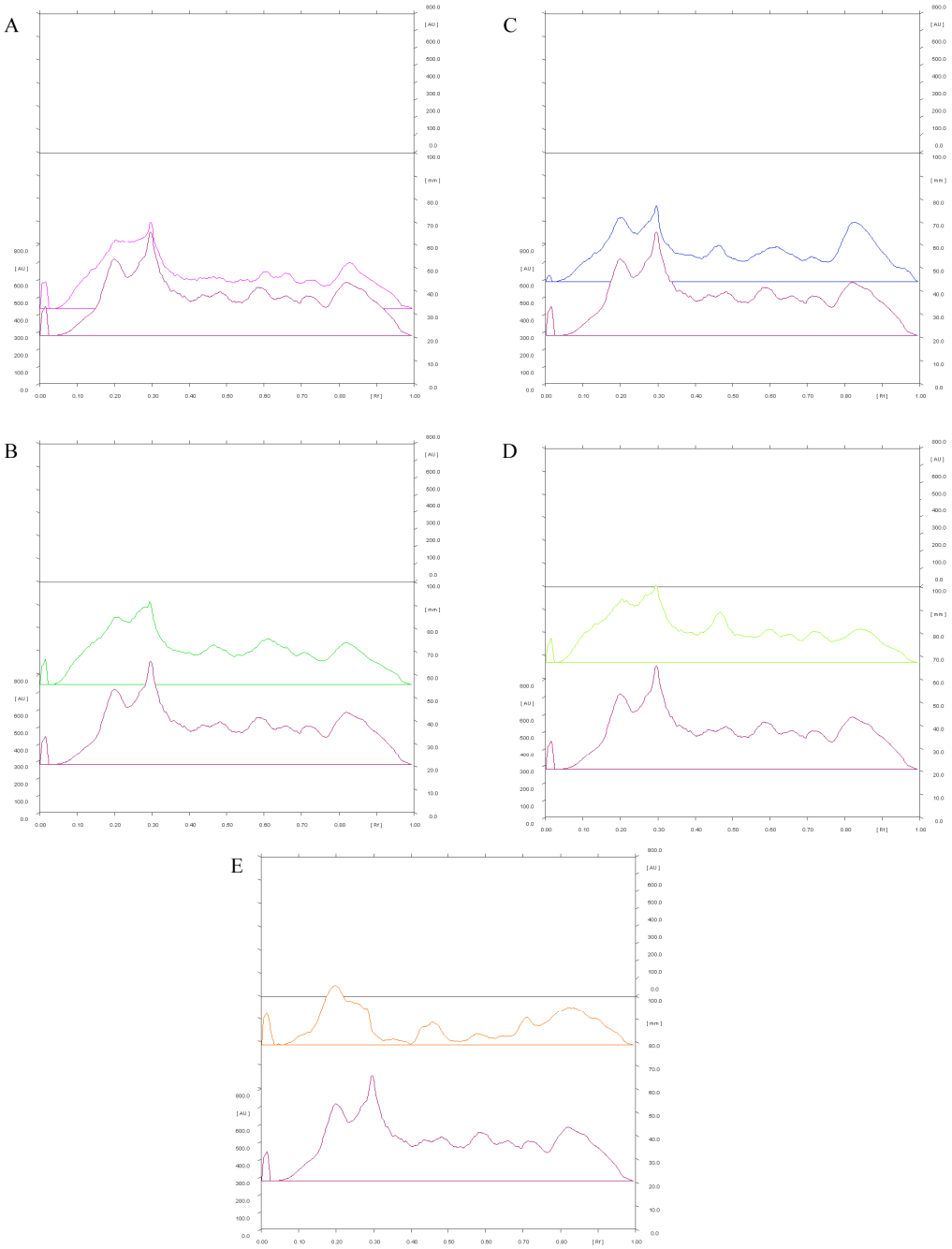


Figure 13. Comparison of dencitometric scan at 580 nm of *Saraca asoca* with A. *Kingiodendron pinnatum*, B. *Cynometra beddomei*, C. *Cynometra travancorica*, D. *Humboldtia brunonis*, E. *Humboldtia vahliana*

3.2. Biological analysis

3.2. A. Plant extracts

3.2. A. a. In vitro antioxidant properties

Superoxide radical scavenging activity

Among the plants studied, the extracts of *Saraca asoca* bark showed most significant superoxide radical scavenging activity. The concentration required for 50% inhibition (IC_{50}) of superoxide generation was 27 and 33 μg/ml for acetone and methanolic extracts, respectively. Among the substitutes, the bark of *Kingiodendron pinnatum* produced remarkable scavenging activity. Fifty percent inhibition of superoxide generation was found at the concentration of 33 μg/ml acetone extract and 30 μg/ml methanolic extract respectively. The other plants including, *Cynometra travancorica* (acetone 80 and methanol 68 μg/ml), *Humboldtia brunonis* (acetone 80 and methanol 35 μg/ml) and *Cynometra beddomei* (acetone 79 and methanol 58 μg/ml) also showed significant superoxide scavenging activity. However, the plant *Humboldtia vahliana* (acetone 200 and methanol 150 μg/ml) did not produce significant scavenging activity. The results are shown in Figure 14 A.

Hydroxyl radical scavenging activity

The degradation of deoxyribose to TBARS by hydroxyl radicals generated from Fe^{3+}/ascorbate/EDTA/H_2O_2 system was markedly decreased by the plant extracts. In the case of *Saraca asoca*, the IC_{50} value was 20 and 30 μg/ml for acetone and methanolic fractions respectively. Among substitutes, the most significant activity (IC_{50} value 25 μg/ml) was found for the methanol extract of *Humboldtia brunonis* bark. At the same time, the acetone extract of this plant showed IC_{50} at the concentration of 52 μg/ml. As in the case of superoxide radical assay, *Humboldtia vahliana* did not produce significant hydroxyl scavenging activity (acetone 160 and methanol 200 μg/ml). All other plants, *Kingiodendron pinnatum* (acetone 60

and methanol 70 µg/ml), *Cynometra travancorica* (acetone 75 and methanol 55 µg/ml), and *Cynometra beddomei* (acetone 95 and methanol 82 µg/ml) also showed significant hydroxyl scavenging activity (Figure 14 B).

Lipid peroxidation

The generation of lipid peroxidation was found to be inhibited by the extracts. The acetone extracts of the bark showed IC_{50} values of 28 µg/ml for *Saraca asoca;* 75 µg/ml for *Kingiodendron pinnatum;* 53 µg/ml for *Cynometra travancorica;* 58 µg/ml for *Cynometra beddomei* and 200 µg/ml for *Humboldtia vahliana*. The activities of the methanolic extracts of the bark showed IC_{50} values of 32 µg/ml for *Saraca asoca;* 68 µg/ml for *Kingiodendron pinnatum;* 23 µg/ml for *Cynometra travancorica;* 44 µg/ml for *Cynometra beddomei* and 200 µg/ml for *Humboldtia vahliana*. The most significant inhibition of lipid peroxidation was obtained with the acetone extract of *Cynometra travancorica* (Figure 14 C).

3.2. A.b. Anti-inflammatory properties

Kingiodendron pinnatum

The sub-plantar injection of carrageenan into the mice hind paw elicited an inflammation and a time-dependent increase in paw edema that was maximal at 3rd hour after carrageenan injection. In the control group, the paw thickness increased by 0.11 mm at the 3rd hour after injection of carrageenan. The inflammatory response to carrageenan induced edema, was significantly reduced by acetone (Table 10) and methanolic (Table 11) extracts of *K. pinnatum* at the doses of 50 and 100 mg/kg body weight given orally 1 hour prior to carrageenan application. The acetone extract given at the above doses reduced the paw edema by 63.6 and 72.7% and respectively, by 3rd hour following carrageenan administration. The 50 and 100 mg/kg body weight methanol extract reduced the paw edema by 45.4 and 63.6%, respectively.

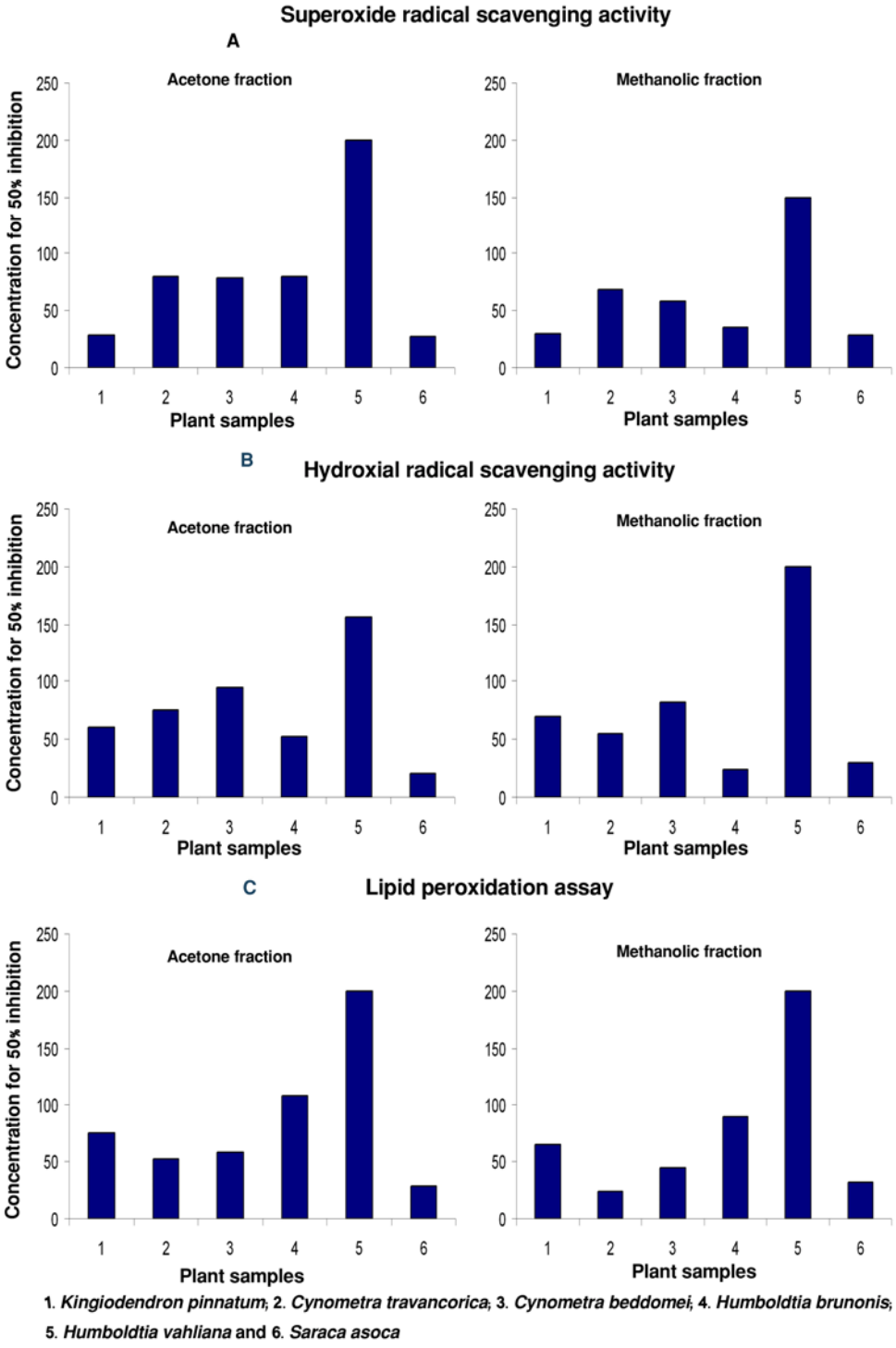


Figure 14. Antioxidant activities of different plant extracts

Cynometra travancorica

The administration of *C. travancorica* leaf and bark methanolic extract at the doses of 50 and 100 mg/kg body weight inhibited the carageenan as well as formalin induced paw edema in a dose-dependent manner. The animals treated with bark extract 50 and 100 mg/kg body weight showed 50 and 61.11% inhibition, respectively of carrageenan induced acute inflammation (Table 12). Similarly, 50 and 100 mg/kg body weight bark extract showed 54.54 and 63.63%, respectively on formalin induced paw edema (Table 13).

Cynometra beddomei

The animals treated with methanolic bark extract of *C. beddomei* at doses of 50 and 100 mg/kg body weight showed 44.44 and 66.66% inhibition of carrageenan induced acute inflammation. In the case of 50 and 100 mg/kg leaf extract treatment showed 55.55 and 77.77% inhibition, respectively on carrageenan induced acute inflammation (Table 14).

The animals treated with methanolic bark of *C. beddomei* extract at doses of 50 and 100 mg/kg body weight showed 54.54 and 63.63% inhibition, respectively on formalin induced inflammation and the leaf extract showed 45.45 and 81.81% inhibition, respectively (Table 15).

Humboldtia brunonis

The administration of *H. brunonis* leaf and bark methanolic extracts to mice was also found to inhibit the paw edema in a dose-dependent manner. The animals treated with methanolic bark extract of *H. brunonis* at doses of 50 and 100 mg/kg body weight showed 44.44 and 66% inhibition of carrageenan induced acute inflammation. Animals treated with methanolic leaf extracts at doses of 50 and 100 mg/kg body weight showed 58.55% and 62% inhibition respectively (Table 16).

On formalin-induced inflammation, the animals treated *H. brunonis* bark extract at doses of 50 and 100 mg/kg body weight showed 50 and 68.18% inhibition, respectively and the leaf extract showed 41.36 and 72% inhibition, respectively (Table 17).

Table 10. Anti-inflammatory activity of bark acetone extract of *Kingiodendron pinnatum* on Carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.28	0.39	0.11	--
Diclofenac	0.29	0.34	0.05	54.0
Acetone (50 mg/kg)	0.26	0.33	0.07	63.6
Acetone (100 mg/kg)	0.26	0.34	0.08	72.7

Table 11. Anti-inflammatory activity of bark methanolic extract of *Kingiodendron pinnatum* on Carrageenan induced paw edema in Swiss albino mice.

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.28	0.39	0.11	--
Diclofenac	0.29	0.34	0.05	54.0
Methanol (50 mg/kg)	0.26	0.32	0.06	45.4
Methanol (100 mg/kg)	0.26	0.30	0.04	63.6

Table 12. Anti-inflammatory activity of methanolic extracts of *Cynometra travancorica* on Carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.220 ± .020	0.310 ± 0.01	0.090	--
Dichlofenac	0.235 ± .017	0.270 ± 0.05	0.040	55.55
Bark, 50 mg/kg body Wt	0.230 ± 0.04	0.275 ± 0.04	0.045	50.00[a]
Bark ,100 mg/kg body Wt	0.230 ± 0.02	0.265 ± 0.04	0.035	61.11[b]
Leaf, 50 mg/kg body Wt	ND	ND		
Leaf, 100 mg/kg body Wt	ND	ND		

Table 13. Anti-inflammatory activity of methanolic extracts of *Cynometra travancorica on* formalin induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	6th day		
Control	0.245 ± 0.01	0.355 ± 0.07	0.110	-----
Dichlofenac	0.265 ± 0.01	0.334 ± 0.03	0.065	40.90
Bark, 50 mg/kg body Wt	0.260 ± 0.02	0.311 ± 0.02	0.050	54.54[a]
Bark, 100 mg/kg body Wt	0.270 ± 0.03	0.312 ± 0.01	0.040	63.63[b]
Leaf, 50 mg/kg body Wt	ND	ND		
Leaf, 100 mg/kg body Wt	ND	ND		

ND-Not Detected, Values are Mean ± SD; for six animals in each group; (a) p, 0.01, (b) $p<0.05$ as compared to control.

Table 14. Anti-inflammatory activity of methanolic extracts of *Cynometra beddomei* on Carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.220 ± 0.02	0.310 ± 0.01	0.09	--
Dichlofenac	0.235 ± 0.02	0.275 ± 0.05	0.04	55.55
Bark, 50 mg/kg body Wt	0.230 ± 0.02	0.280 ± 0.05	0.05	44.44[a]
Bark, 100 mg/kg body Wt	0.240 ± 0.03	0.270 ± 0.02	0.03	66.66[b]
Leaf, 50 mg/kg body Wt	0.220 ± 0.01	0.26 ± 0.02	0.04	55.55[a]
Leaf, 100 mg/kg body Wt	0.240 ± 0.08	0.26 ± 0.008	0.02	77.77[b]

Table 15. Anti-inflammatory activity of methanolic extracts of *Cynometra beddomei* on formalin induced paw odema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	6th day		
Control	0.245 ± 0.01	0.355 ± 0.24	0.110	--
Dichlofenac	0.265 ± 0.12	0.330 ± 0.03	0.065	40.90
Bark, 50 mg/kg body Wt	0.260 ± 0.02	0.310 ± 0.02	0.050	54.54[a]
Bark, 100mg/kg body Wt	0.270 ± 0.03	0.310 ± 0.01	0.040	63.63[b]
Leaf, 50 mg/kg body Wt	0.280 ± 0.02	0.340 ± 0.03	0.06	45.45[a]
Leaf, 100 mg/kg body Wt	0.240 ± 0.08	0.260 ± 0.09	0.02	81.81[b]

Values are Mean ± SD; for six animals in each group; (a) p, 0.01, (b) $p<0.05$ as compared to control

Table 16. Anti-inflammatory activity of methanolic extracts of *Humboldtia brunonis* on carageenan induced paw odema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.220 ± 0.02	0.310 ± 0.01	0.09	--
Dichlofenac	0.235 ± 0.02	0.275 ± 0.05	0.04	55.55
Bark, 50 mg/kg body Wt	0.230 ± 0.02	0.280 ± 0.05	0.05	44.44[a]
Bark, 100 mg/kg body Wt	0.240 ± 0.03	0.270 ± 0.02	0.03	66.66[b]
Leaf, 50 mg/kg body Wt	0.230 ± 0.03	0.268 ± 0.04	0.038	58.55[a]
Leaf, 100 mg/kg body Wt	0.205 ± 0.08	0.24 ± 0.008	0.035	62.00[b]

Table 17. Anti-inflammatory activity of methanolic extracts of *Humboldtia brunonis* on formalin induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	6th day		
Control	0.245 ± 0.01	0.355 ± 0.07	0.110	--
Dichlofenac	0.265 ± 0.01	0.330 ± 0.03	0.065	40.90
Bark, 50 mg/kg body Wt	0.240 ± 0.02	0.295 ± 0.02	0.055	50.00
Bark, 100 mg/kg body Wt	0.245 ± 0.02	0.280 ± 0.02	0.035	68.18
Leaf, 50 mg/kg body Wt	0.268 ± 0.04	0.332 ± 0.04	0.064	41.36
Leaf, 100 mg/kg body Wt	0.258 ± 0.03	0.288 ± 0.04	0.030	72.00

Values are Mean ± SD; for six animals in each group; (a) p, 0.01, (b) $p<0.05$ as compared to control.

3.2. A. c. Effects on estrogen induced keratinization

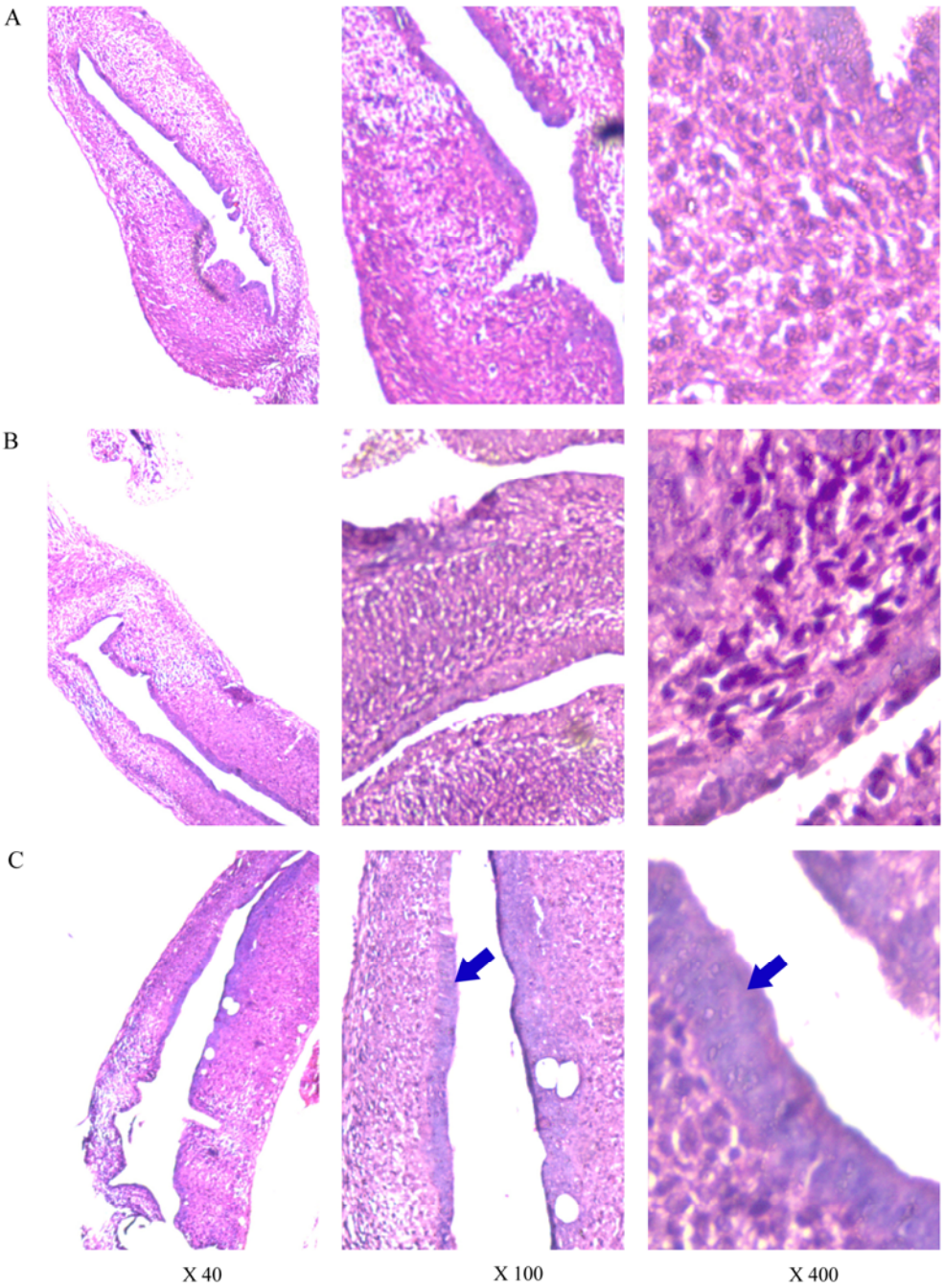
Administration of methanolic extract of *S. asoca* and the substitutes *K. pinnatum* and *C. travancorica* was found to be effective in preventing keratinization of uterus epithelium in Wistar rats. From the histopathological observations, estradiol treated group showed strong evidence of keratinization which extended to 3-4 layers with vacuolated keratinized cells in the outermost layer (epithelia) of endometrium, where as there was no evidence for keratinization in control (without treatment) and propylene glycol (vehicle control) (Plate 3) after 8th day of birth. In the *S. asoca* treated group also, there was evidence of keratinization at endometrium. But the keratinization was limited only to 2 layers in the edometrium and myometrium remained normal. In the case of *K.*

pinnatum treated animals, some areas show keratinization which was also limited to inner 2 layers in endometrium. In *C. travancorica* treated animals, keratinization was limited to 2-3 layers. In all the groups, keratinization was limited in uterus epithelium and there was no evidence of thickening at perimetrium and endometrial glands (Plate 4).

At 16th day prominent layers of keratinized cells were evident in the estradiol treated group, whereas in most of the animals in propylene glycol and drug treated groups there was evidence of thin layer of keratinization with normal endometrium lined by columnar cells throughout. The myometrium appeared to be normal, especially in *S. asoca* treated group. A thin layer of cornified thickening was observed in *Kingiodendron* treated animals. But in *C. travancorica* treated animals, keratinization was limited to 2-3 layers of enodmetrium (Plates 5 and 6). Keratinized columnar cells at the boarder of endometrial gland were also observed in this group.

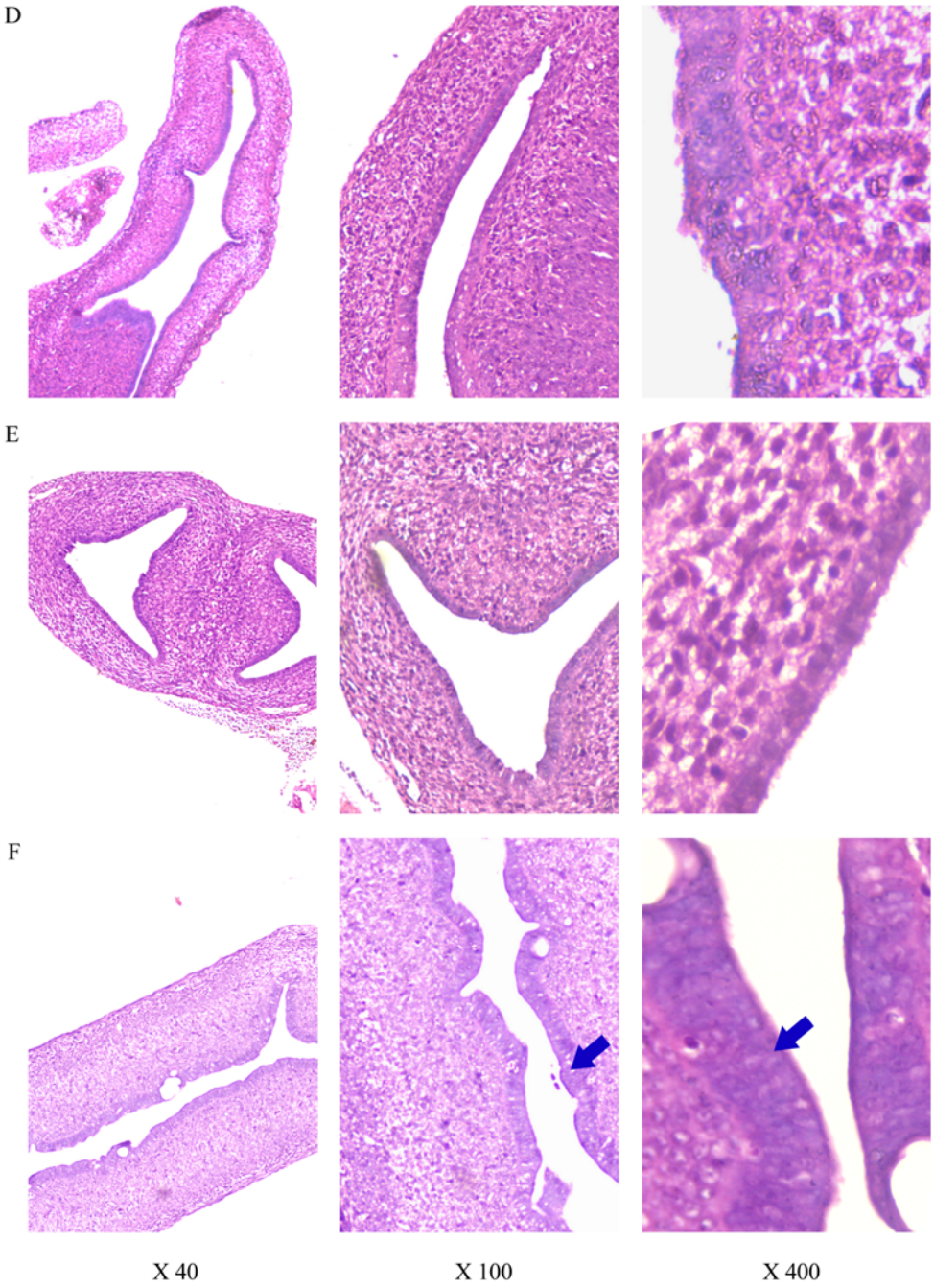
At 24th day, the uterus appeared as normal in the control and propylene glycol treated groups while in the estradiol groups there were prominent layers (4-6 layers of cornification) of keratinized epithelial cells (Plates 7 and 8). In this group, well keratinized perimetrium and keratinized columnar cells at endometrium were also noticed. Even though the keratinized endometrial layers were less evidenced in *S. asoca* treated group, well keratinized columnar endometrial glands were distinct. In *C. travancorica* treated groups, both endometrial keratinized epithelial as well as columnar gland cells were produced.

As shown in the Plates 9 to 12, the intensity of keratinization got diminished after 32 days of treatment. Two or three layers of keratinized epithelia were noticed in most of the groups including estrogen alone treated groups.



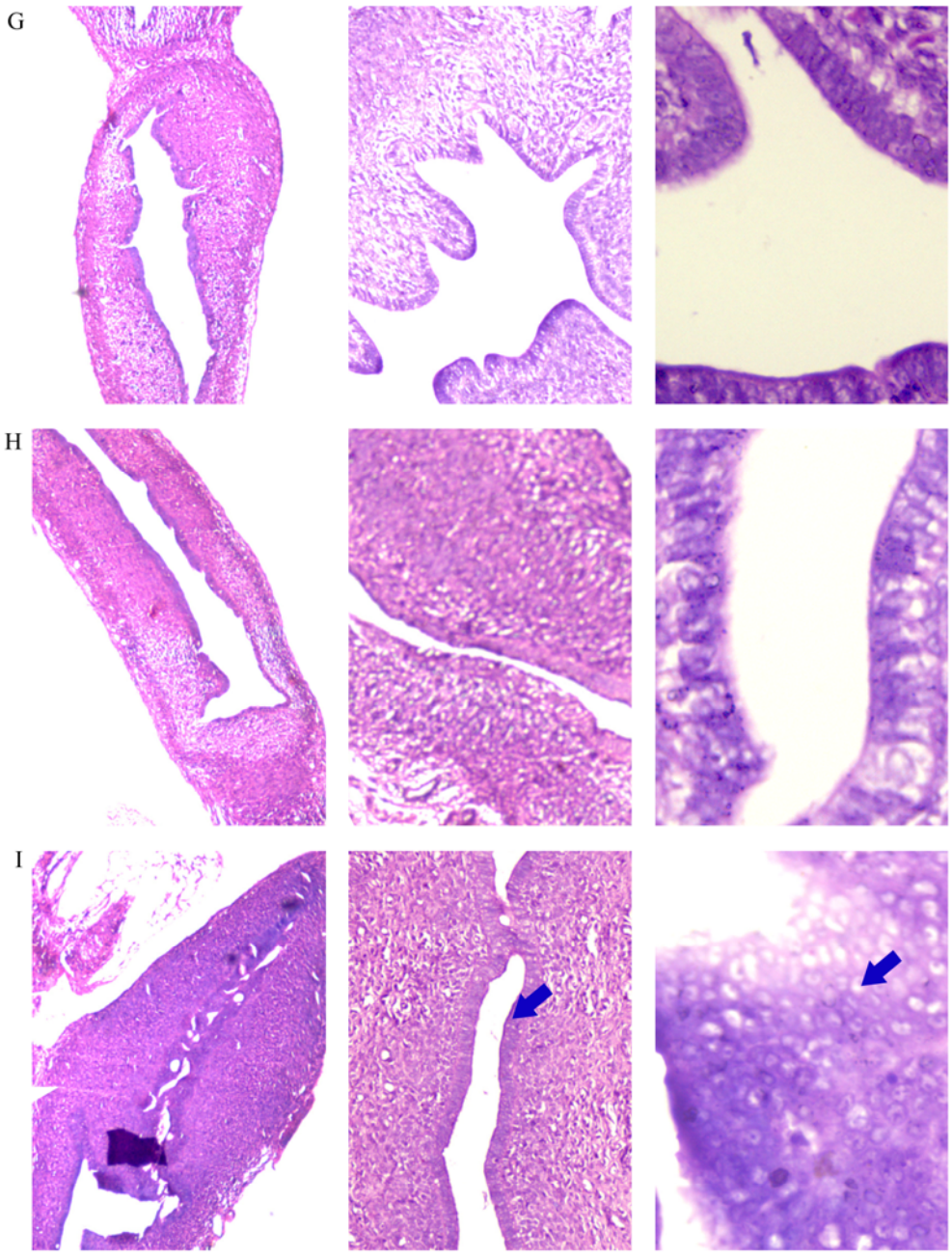
Photomicrogrpah of a portion of the uterous of rat after 8 th day of treatment. The parafin wax embedded microtome sections were stained with hematoxylin and eosin for the detection of keratinized epithelium. A. Control, B. Propanediol treated and C. Estradiol traeted group. The area of cornified epithelium in edometrium is marked with arrow.

PLATE 3



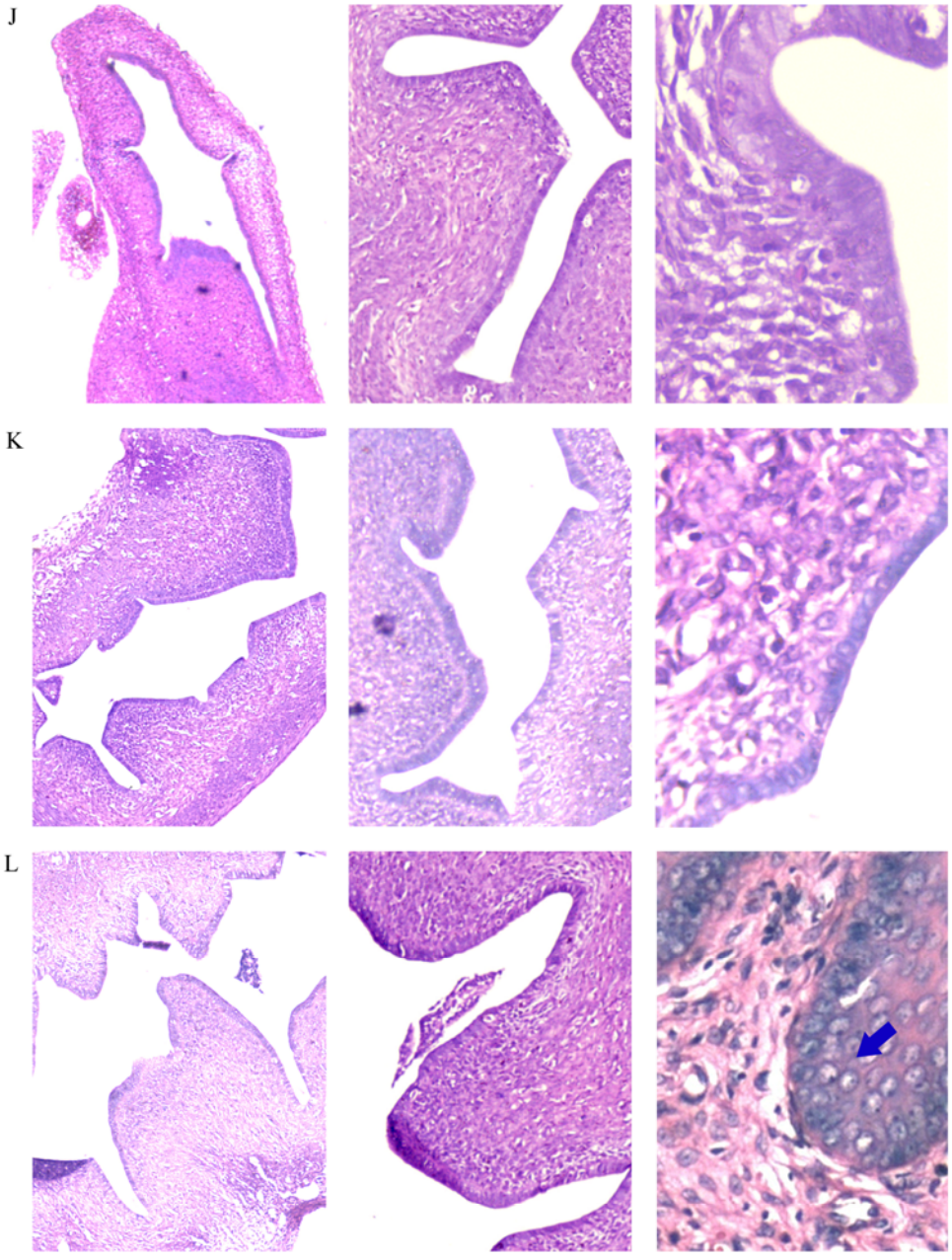
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 8th day of treatment. The parafin wax embedded microtome sections were used. D, Estradiol with *Saraca asoca*; E. Estradiol with *Kingiodendron pinnatum* and F, Estradiol with *Cynometra travancorica*.

PLATE 4



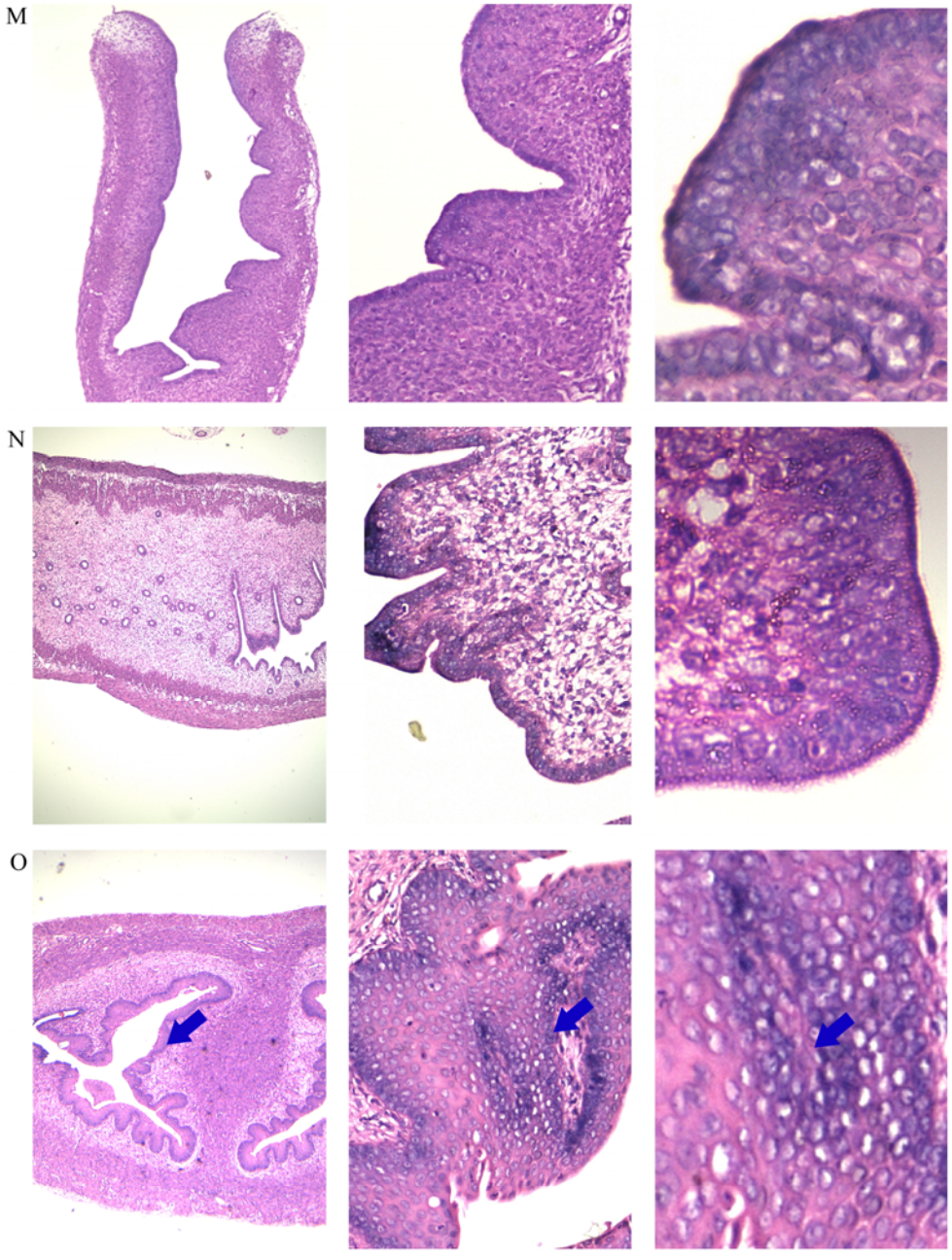
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 16th day of tretment. The parafin wax embedded microtome sections were used. G. Control, H. Propanediol treated, I. Estradiol traeted.

PLATE 5



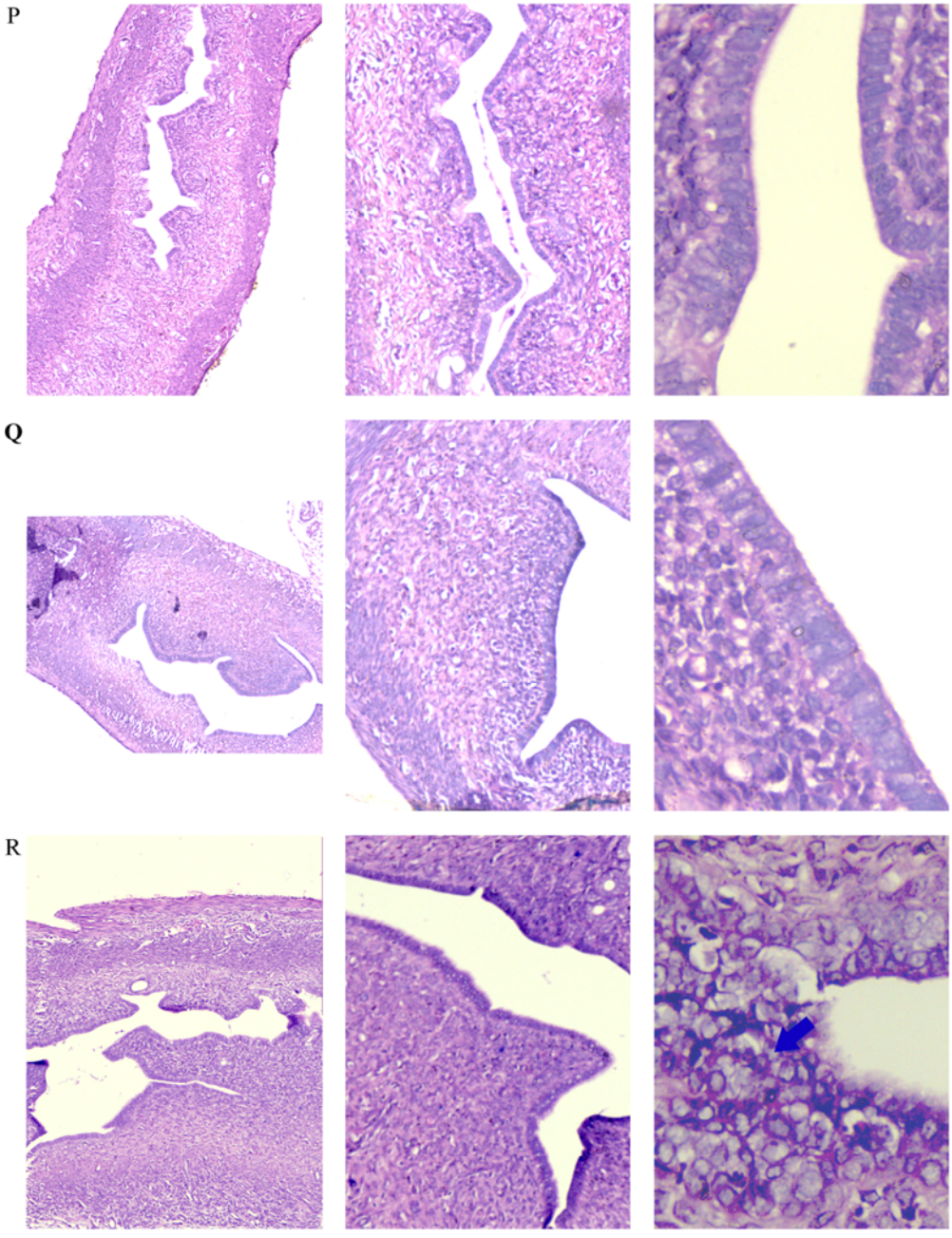
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 16th day of treatment. The parafin wax embedded microtome sections were used. J, Estradiol with *Saraca asoca*; K. Estradiol with *Kingiodendron pinnatum* and L, Estradiol with *Cynometra travancorica.*

PLATE 6



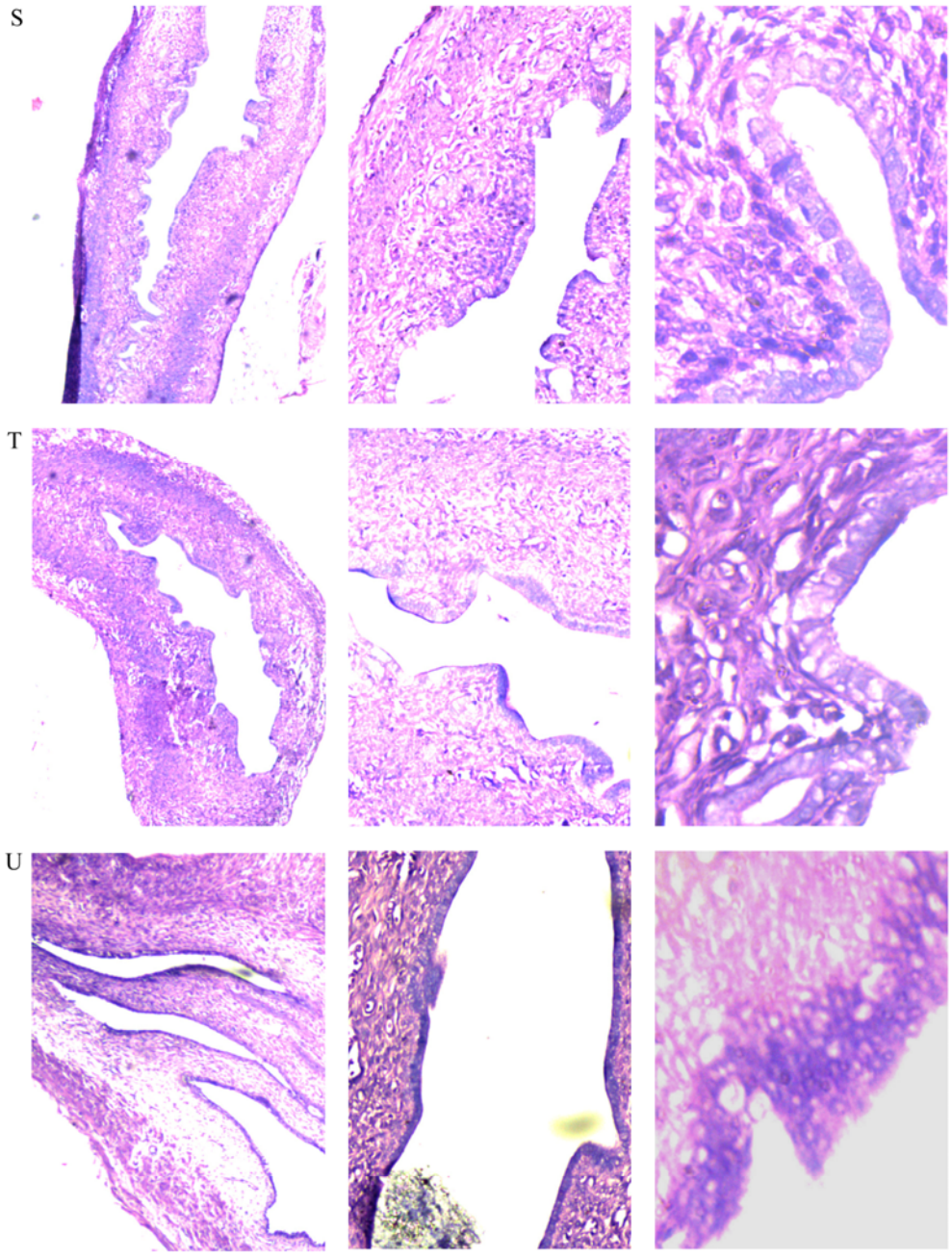
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 24th day of treatment. M. Control, N. Propanediol treated and O. Estradiol traeted.

PLATE 7



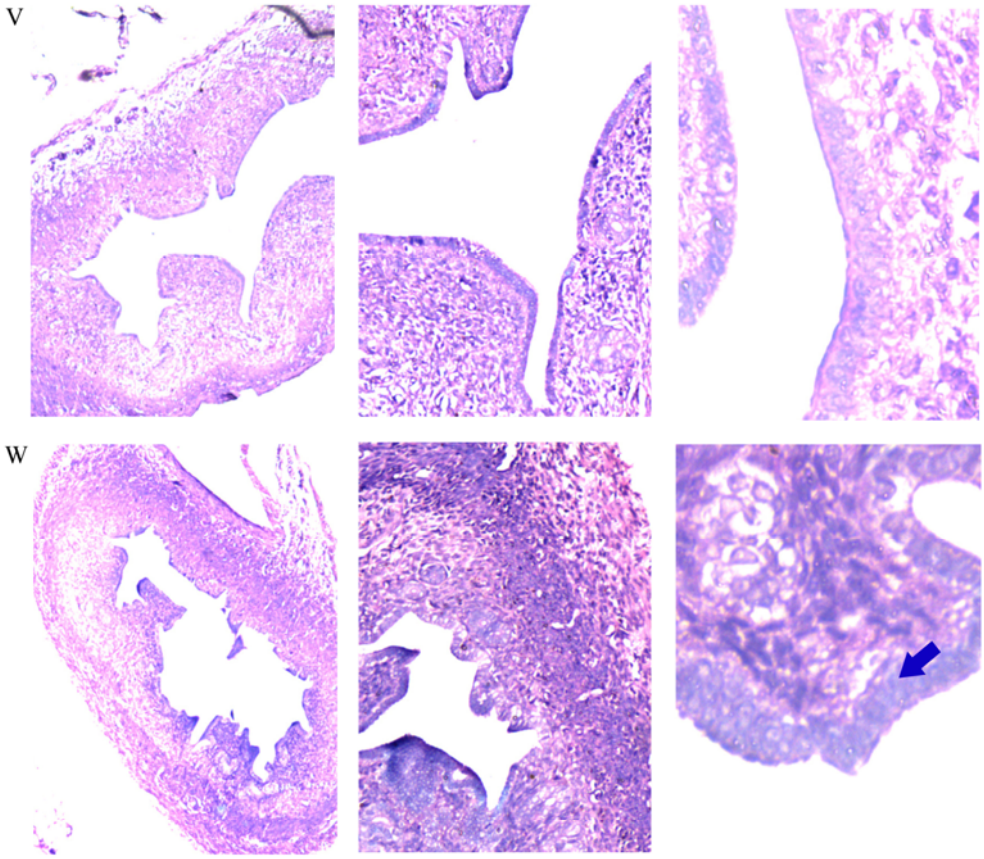
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 24th day treatment. P, Estradiol with *Saraca asoca*; Q. Estradiol with *Kingiodendron pinnatum* and R, Estradiol with *Cynometra travancorica*.

PLATE 8



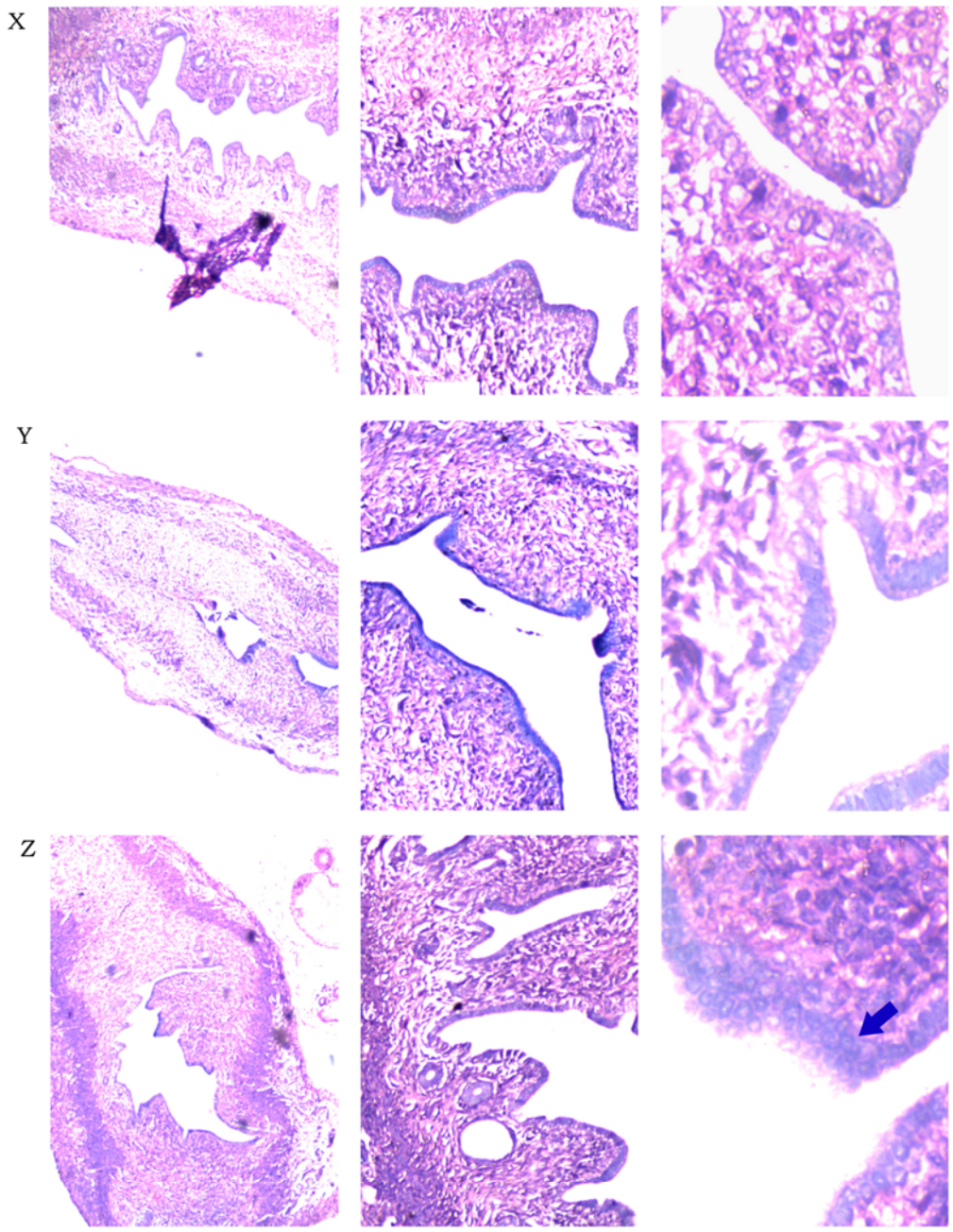
Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 32nd day treatment. The parafin wax embedded microtome sections were used. S. Control, T. Propanediol treated, U. Estradiol traeted

PLATE 9



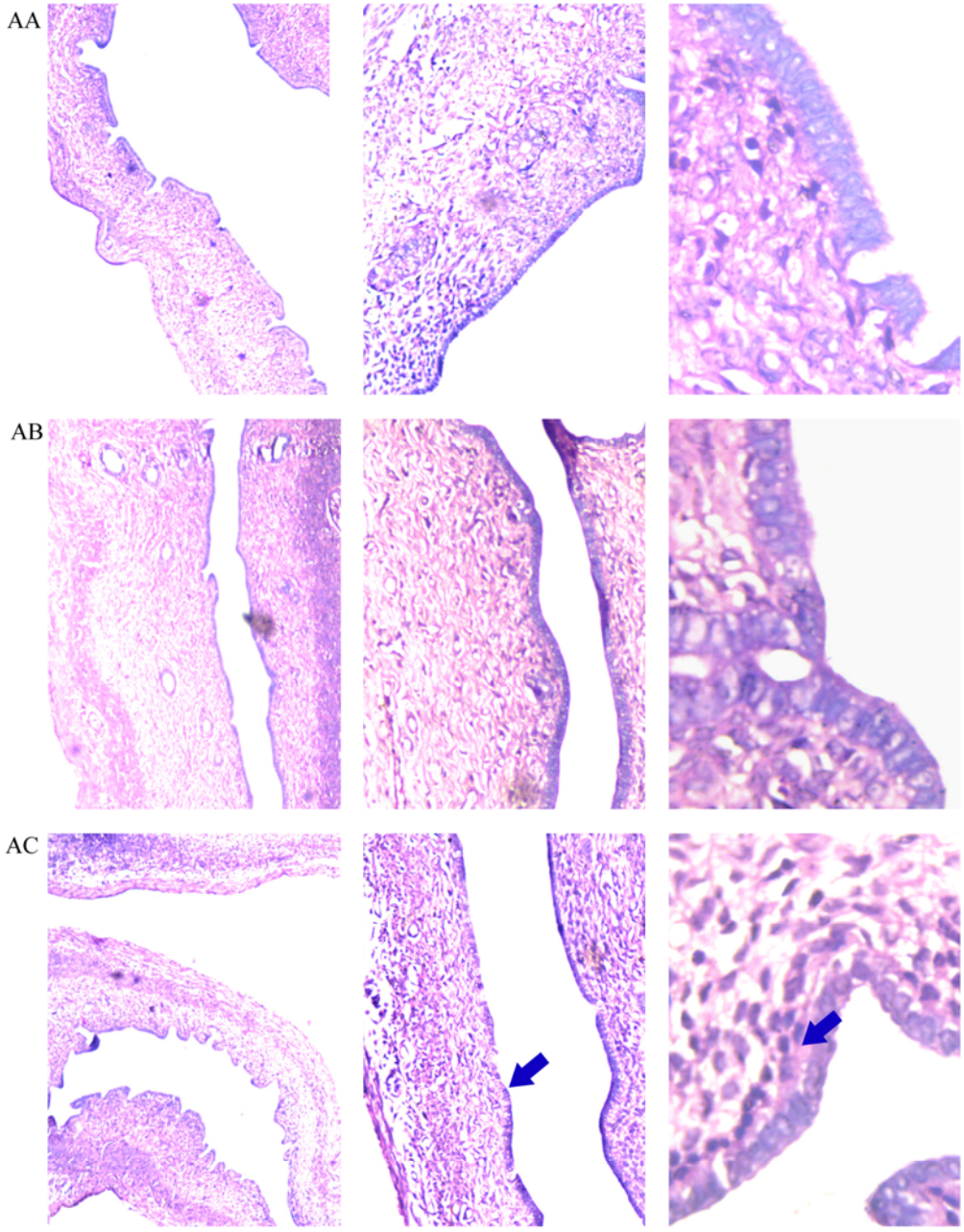
Photomicrogrpah of a portion of the uterus of rat after 32nd day of treatment. The parafin wax embedded microtome sections were stained with hematoxylin and eosin for the detection of keratinized epithelium. V, Estradiol with *Saraca asoca*; W. Estradiol with *Cynometra travancorica*.

PLATE 10



Photomicrogrpah of a portion of the uterous of rat after 40 th day of treatment. The parafin wax embedded microtome sections were stained with hematoxylin and eosin for the detection of keratinized epithelium. X. Control, Y. Propanediol treated, Z. Estradiol traeted group. The area of cornified epithelium in edometrium is marked with arrow.

PLATE 11



Photomicrogrpah of a portion of the uterous of rat stained with hematoxylin and eosin for the detection of keratinized epithelium after 40th day of treatment. The parafin wax embedded microtome sections were used. Estradiol with *Saraca asoca*; Estradiol with *Kingiodendron pinnatum* and Estradiol with *Cynometra travancorica.*

PLATE 12

3.2.A.d. Effects on antiestrogenic properties

As shown in the Table 18, *Saraca asoca* and its substitutes possessed significant antiestrogenic activity. The estrogen level in the untreated control group was 111.2 U/L. The level is reduced to 82.9, 77.5 and 56.2 U/L in groups treated with extracts of *S. asoca*, *K. pinnatum* and *C. travancorica* respectively. Among the substitutes, *C. travancorica* treated group showed significant antiestrogenic activity.

Table 18. Estrogen level in blood of treated rats analysed by RIA

Sl. No	Groups	Estrogen (U/L)
1	Control	111.2 ± 30.08
2	*S. asoca*	82.9 ± 32.88
3	*K. pinnatum*	77.5 ± 49.29
4	*C. travancorica*	56.2 ± 29.51

Hemoglobin level

Approximately, 10 μl of blood was mixed with 2.5 ml of Drabkins reagent, mixed well and kept for 5 minutes and read at 546 nm using spectrophotometer (Table 19).

Table 19. Hemoglobin level of treated rats by Drabkins method

Sl. No	Groups	Hb g/dl
1	Control	14.0 ± 2.57
2	*S. asoca*	14.0 ± 1.32
3	*K. pinnatum*	13.4 ± 1.86
4	*C .travancorica*	15.3 ± 0.80

Leucocyte count

Total leucocyte count was measured by hemocytometer (Table 20).

Table 20. Total leucocyte count of treated rats

Sl. No	Groups	Total WBC count
1	Control	6850 ± 1660
2	*S. asoca*	7937 ± 0670
3	*K. pinnatum*	7075 ± 1274
4	*C. travancorica*	6700 ± 0932

Differential count

The percentage level of lymphocyte, monocyte, neutrophil, basophil and eosinophil were determined microscopically by staining with leishman stain (Table 21 and Plate 13).

Table 21. The effect of plant extracts on percentage of polymorphoneutrophil (PMN)

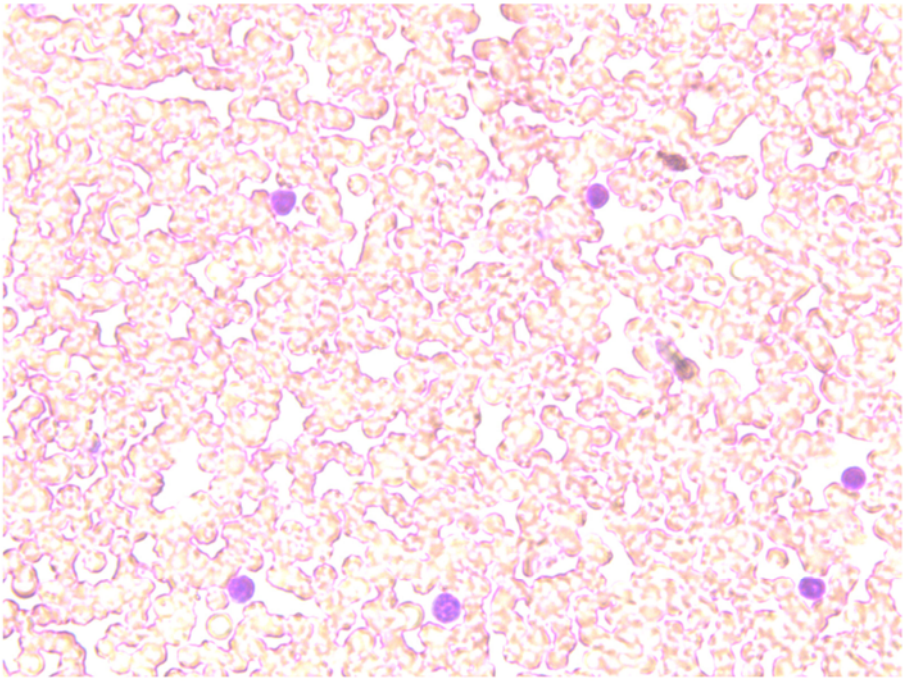
Sl.No.	Groups	Lymphocyte	Monocyte	Neutrophil	Basophil	Eosinophil
1	Control	70 ± 6.0	12 ± 3.5	10 ± 4.40	02 ±1	04 ± 2.0
2	*S. asoca*	67 ± 3.0	11 ±1.8	11 ± 1.70	03 ±1	07 ± 2.8
3	*K. pinnatum*	60 ±18.9	15 ±1.2	11 ± 5.50	04 ±1	06 ± 6.0
4	*C. travancorica*	61 ±13.3	12 ± 4.8	15 ± 9.53	04 ±1	07 ±1.6

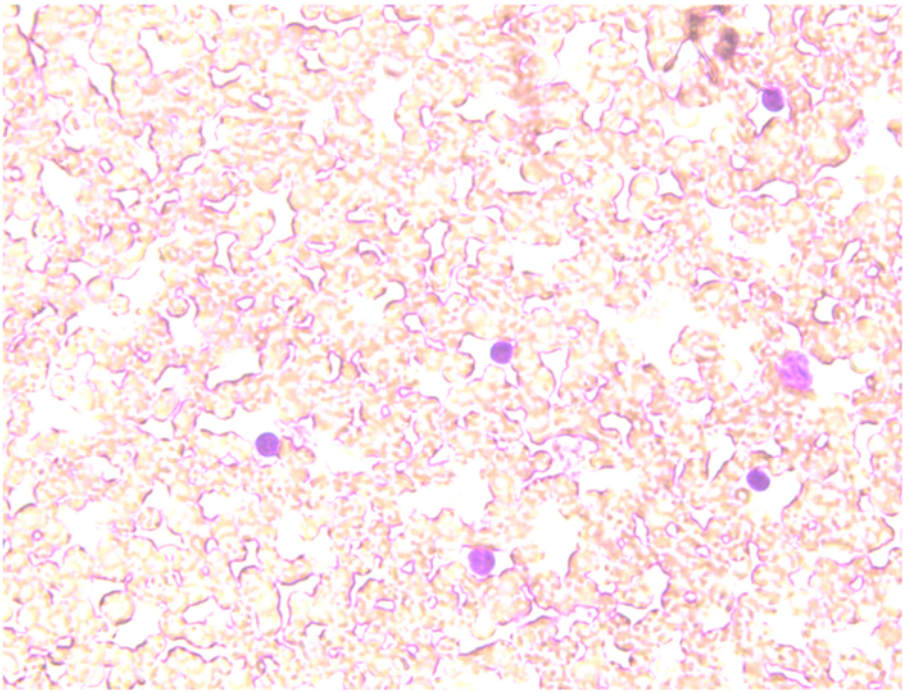
Body weight of the animals was also recorded (Table 22).

Table 22. Body weight of animals treated by extracts

Sl. No	Groups	Body wt (in gm) 1st day	Body wt (in gm) 10th day
1	Control	197 ± 16.80	197 ±12.18
2	*S. asoca*	209 ± 08.99	205 ±12.12
3	*K. pinnatum*	199 ± 12.80	197 ± 09.09
4	*C. travancorica*	189 ± 14.60	186 ± 08.28

Compared to control groups, the hemoglobin level, total and differential leucocyte counts of experimental groups were found to be normal. This indicates that the *Saraca asoca* and its substitutes are non toxic to experimental animals.





Differential Leucocyte count (DC)

PLATE 13

3.2. B. Ayurvedic preparations (Arishtams)

3.2. B. a. In vitro antioxidant properties

Hydroxyl radical scavenging activity

The degradation of deoxyribose to TBARS by hydroxyl radicals generated from Fe3+/ascorbate/EDTA/H_2O_2 system got decreased markedly by the plant extracts. The value for Arishtam prepared with *S. asoca, K. pinnatum, C. travancorica, H. brunonis* and *P. longifolia* were 5, 4.5, 4.5, 5 and 23.5 µl respectively (Figure 15).

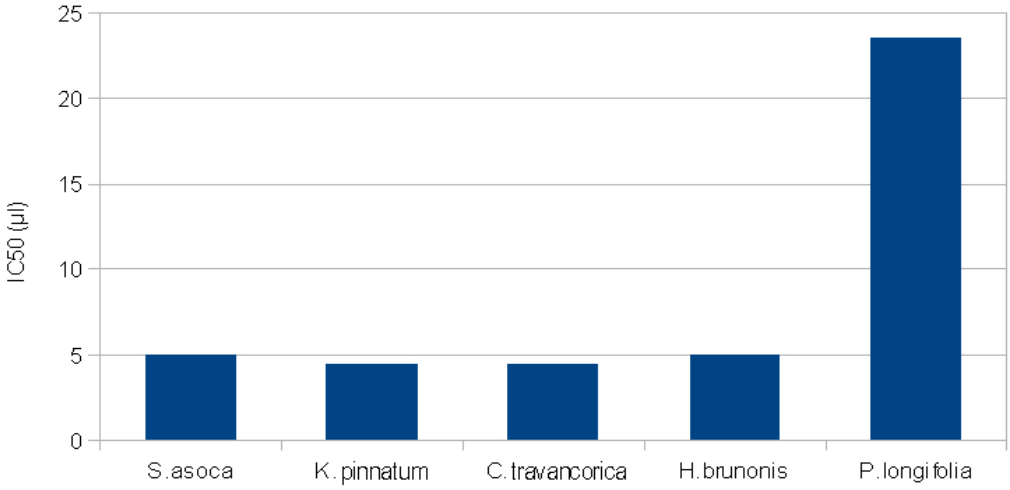


Figure 15. Concentration needed for 50 % inhibition of hydroxyl radical for arishtam prepared with *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia*

Inhibition of lipid peroxidation

The capacity of extracts to prevent lipid peroxidation was assayed using malondialdehyde formation as an index of oxidative breakdown of membrane lipids, following incubation of rat liver homogenate with the oxidant chemical species Fe. Among *Saraca asoca* and its substitutes, the former showed more effective activity in preventing lipid peroxidation with an IC50 value of 7.5 *µl.* The IC50 value of *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia* were 20, 21, 22 and 27 µl respectively (Figure 16).

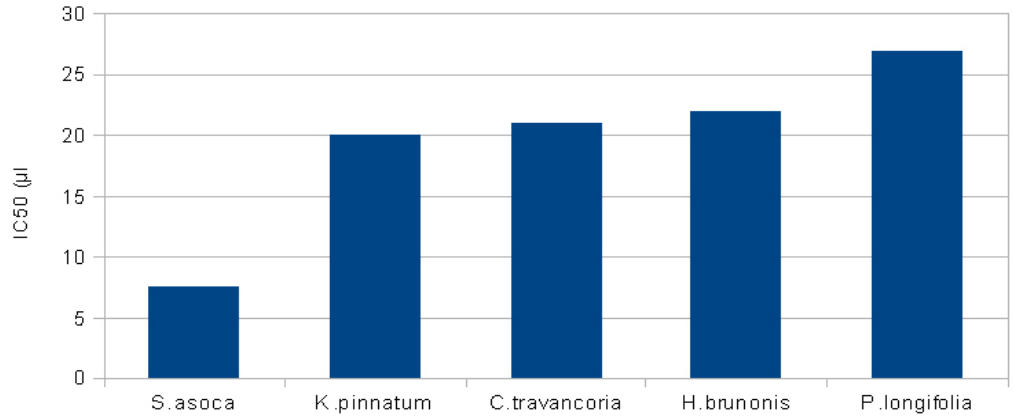


Figure 16. Concentration needed for 50 % inhibition of lipid peroxidation for arishtam prepared with *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia*

DPPH radical scavenging activity

Among the Arishtams prepared with *Saraca asoca* and its substitutes, *Humboldtia brunonis* efficiently scavenged the stable free radical DPPH (2, 2 diphenyl – 1- picryl hydrazyl) and the IC50 value of the formulation was found to be 0.3 µl. The IC50 values of *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia* were 7.5, 1.35, 0.6 and 0.4 µl, respectively (Figure 17).

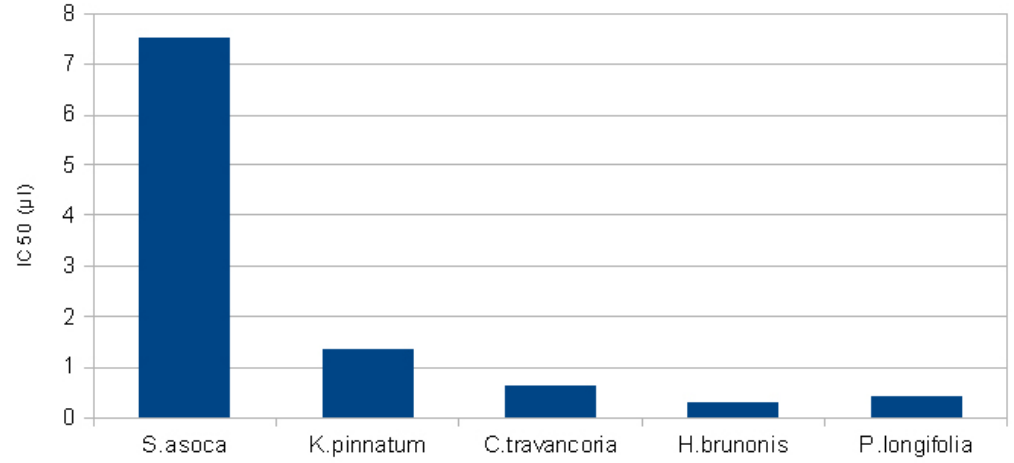


Figure 17. Concentration needed for 50 % inhibition of DPPH radical for arishtam prepared with *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia*

ABTS scavenging activity

Arishtam prepared with *Kingiodendron pinnatum* showed good ABTS radical scavenging activity among all other preparations, the IC50 value of the formulation was found to be 0.15µl. The IC50 values of *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia* were 0.3, 0.375, 0.375 and 0.175 µl respectively (Figure 18).

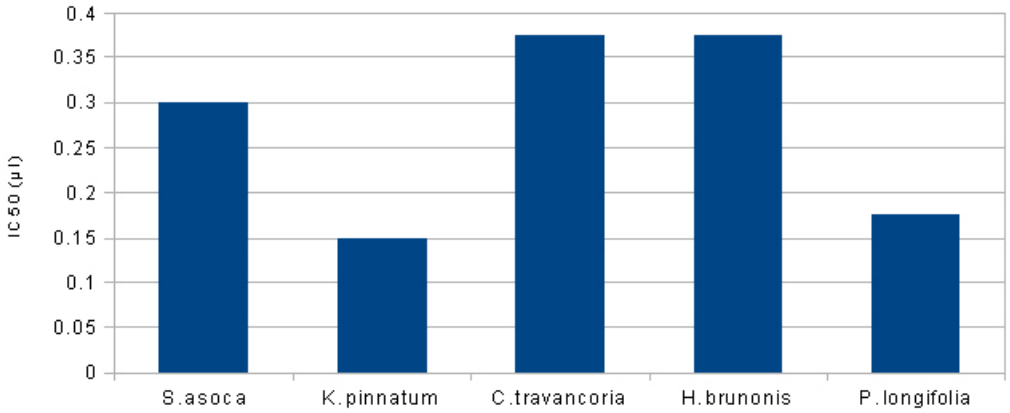


Figure 18. Concentration needed for 50 % inhibition of ABTS radical for arishtam prepared with *S. asoca, K. pinnatum, C. travancorica, H. brunonis and P. longifolia*

FRAP assay

Total antioxidant activity of an extract is measured by ferric reducing antioxidant power assay (FRAP) of Benzie and Strain (1999). FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method employing an easily reduced oxidant system present in stoichiometric excess.

Saraca asoca

Ferric reducing activity of 1µl methanolic extract of *S. asoca* is equivalent to the reducing power of 0.24 mM FeSO4.7H2O

Kingiodendron pinnatum

Ferric reducing activity of 5µl methanolic extract of *K. pinnatum* is equivalent to the reducing power of 0.36 mM FeSO4.7H2O

Cynometra travancorica

Ferric reducing activity of 4 µl methanolic extract of *C. travancorica* is equivalent to the reducing power of 0.20 mM FeSO4.7H2O.

Humboldtia brunonis

Ferric reducing activity of 4 µl methanolic extract of *H. brunonis* is equivalent to the reducing power of 0.34 mM FeSO4.7H2O.

Polyalthia longifolia

Ferric reducing activity of 1µl methanolic extract of *P. longifolia* is equivalent to the reducing power of 0.22mM FeSO4.7H2O.

3.2. B. b. *In vivo* antioxidant properties

Effect of prepared Arishtams on antioxidant enzymes and glutathione in blood and liver of mice after administration for a period of 30 days is shown in Table 23. Catalase activity got significantly increased in Arishtam treated groups at the concentration of 400 µl/animal. The percentage in the increase of activity was found to be 33.17, 36.87 and 49.18% with Arishtams prepared with *S. asoca, K. pinnatum* and *C. travancorica*, respectively. In the animals treated with 200 µl, the percentage increase was 10.09, 36.87 and 15.92 respectively.

The activity of superoxide dismutase also increased by 16.89 and 31.33% in the groups treated with 200 and 400 µl of Arishtams prepared with *S. asoca*. In the groups treated with Arishtams prepared with *K. pinnatum,* the increase was 24.43 and 39.05% and for *C. travancorica* the increase was 27.91 and 48.58% when compared to the untreated control groups. Glutathione was found to be significantly elevated in 200 (25.98%) and 400 µl/animal (51.69%) with *Saraca asoca* Arishtam. Arishtams with *K. pinnatum* showed 19.21 and 43.76% and *C. travancorica* showed 9.75 and 56.36% of increase for 200 and 400 µl/animal respectively.

Effect of Arishtams on the antioxidant enzymes in mice liver after treatment for 30 days is given in Table 24. The catalase activity increased in groups treated with arishtam prepared with *Saraca asoca* by 16.48 and 20.06% for doses 200 and 400 µl/animal when compared to the untreated control group. Arishtam with *K. pinnatum* showed 4.68 and 16.79% and *C. travancorica* showed 12.43 and 16.17% of increase respectively.

Glutathione peroxidase also increased in the Arishtam treated groups at the concentration of 200 µl/animal. The percentage increase of activity was found to be 19.67 22.52 and 28.71 % with Arishtams prepared with *S. asoca, K. pinnatum, C. travancorica* and *H. brunonis* respectively. At the concentration of 400 µl the percentage increase was 28.61, 27.12 and 35.28%, respectively. Glutathione increased by 8.23 and 32.32% in the group treated with Arishtam prepared with *S. asoca;* 6.94 and 10.66% for *K. pinnatum* and 14.1 and 30.93% for *C. travancorica,* compared to untreated control groups.

Table 23. Effect of prepared Arishtams on antioxidant enzymes in the blood

Treatment	Catalase (K/g Hb)	Superoxide dismutase (U/g Hb)	Glutathione (nmols/ml)
Normal	80.75±16.73	910.52±52.2	23.13±2.25
S. asoca (200 µl)	100.9±15.41 (+ 19.97)	1095.55±82.61 (+ 16.89)	31.25±2.63 (+ 25.98)
S. asoca (400 µl)	120.83±15.76 (+ 33.17)	1326.02±58.09 (+ 31.33)	47.88±7.04 (+ 51.69)
K. pinnatum (200 µl)	89.81±28.72 (+10.09)	1204.93±33.64 (+ 24.43)	28.63±4.75 (+ 19.21)
K. pinnatum (400 µl)	127.91±26.58 (+ 36.87)	1493.78±55.74 (+39.05)	41.13±5.54 (+ 43.76)
C. travancorica (200 µl)	96.04±15.51 (+15.92)	1262.32±66.05 (+ 27.91)	25.63±1.65 (+ 9.75)
C. travancorica (400 µl)	158.88±19.34 (+ 49.18)	1770.61±88.43 (+ 48.58)	53±1.68 (+ 56.36)

k = the measure of catalase activity (the difference in extinction at 240 nm per 15 seconds)

Table 24. Effect of prepared Arishtams on antioxidant enzymes in the tissue

Treatment	Catalase (K/g Hb)	Glutathione Peroxidase (U/g Hb)	Glutathione (nmols/ml)
Normal	11.2±0.46	16.82±2.6	6.7±2.34
S. asoca (200 µl)	13.41±3.35 (+16.48)	20.94±3.85 (+ 19.67)	7.1±1.78 (+8.23%)
S. asoca (400 µl)	14.01±2.07 (+ 20.06)	23.56±3.85 (+ 28.61)	9.9±1.43 (+ 32.32)
K. pinnatum (200 µl)	11.75±2.02 (+ 4.68)	21.71±4.93 (+ 22.52)	6.9±1.3 (+6.94%)
K. pinnatum (400 µl)	13.46±2.18 (+ 16.79)	23.08±4.85 (+ 27.12)	7.5±1.77 (+ 10.66)
C. travancorica (200 µl)	12.79±1.14 (+ 12.43)	23.6±2.37 (+ 28.71)	7.4±1.26 (+14.1%)
C. travancorica (200 µl)	13.36±2.54 (+ 16.17)	25.99±4.57 (+ 35.28)	9.7±0.68 (+ 30.93)

k = the measure of catalase activity (the difference in extinction at 240 nm per 15 seconds)

3.2.B.c. Anti-inflammatory studies

Arishtam prepared with S. asoca

The sub-plantar injection of carrageenan into the mice hind paw elicited an inflammation and a time-dependent increase in paw edema that was maximal at 3rd hour after carrageenan injection. In the control group, the paw thickness increased by 0.363 mm at the 3rd hour after injection of carrageenan. The inflammatory response to carrageenan induced edema, was significantly reduced by Asokarishtam (Table 25) at the doses of 200 and 400 µl/kg body weight given orally 1 hour prior to carrageenan application. The Arishtam given at the above doses reduced the paw edema by 49.41 and 65.8% respectively, by 3rd hour following carrageenan administration. On formalin induced inflammation, thickens of paw edema increased to 0.330 by 3rd day of formalin administration. The Arishtam at 200 and 400 µl/kg body weight concentration reduced the paw edema by 32.87 and 52.13% respectively (Table 26).

Arishtam prepared with K. pinnatum

The administration of Aristam prepared with *C. travancorica* inhibited the carageenan as well as formalin induced paw edema in a dose dependent manner. The animals treated with 200 and 400 µl/kg body weight showed 55.2 and 60.0% inhibition, respectively of carrageenan induced acute inflammation (Table 27). Similarly, Arishtam showed 48.56 and 52.59% inhibition respectively on formalin induced paw edema (Table 28).

Arishtam prepared with C. travancorica

The animals treated with Arishtam prepared with *C. travancorica* at doses of 200 and 400 µl/kg body weight showed 55.29 and 67.06% inhibition of carrageenan induced acute inflammation (Table 29). The Aristam at the doses of 200 and 400 µl/kg body weight showed 46.71 and 53.52% inhibition, respectively on formalin induced inflammation (Table 30).

Table 25. Effect of Asokarishtam on carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.218 ± .021	0.363 ± 0.07	0.145	--
Dichlofenac	0.216 ± .016	0.260 ± 0.09	0.044	69.41
S. asoca 200 µl/kg bwt	0.215 ± 0.08	0.288 ± 0.24	0.073	49.41
S. asoca 400 µl/kg bwt	0.220 ± 0.12	0.270 ± 0.14	0.050	65.80

Table 26. Effect of Asokarishtam on formalin induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0th day	3rd day		
Control	0.194 ± 0.15	0.330 ± 0.09	0.136	--
Dichlofenac	0.212 ± 0.28	0.269 ± 0.18	0.057	58.02
S. asoca 200 µl/kg b.wt	0.212 ± 0.07	0.304 ± 0.14	0.092	32.87
S. asoca 400 µl/kg b.wt	0.214 ± 0.31	0.279 ± 0.29	0.065	52.13

Table 27. Effect of Aristam prepared with *K. pinnatum* on carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.218 ± .021	0.363 ± 0.07	0.145	--
Dichlofenac	0.216 ± .016	0.260 ± 0.09	0.044	69.41
K. pinnatum 200 µl/kg bwt	0.215 ± 0.05	0.290 ± 0.14	0.065	55.2
K. pinnatum 400 µl/kg bwt	0.210 ± 0.32	0.268 ± 0.24	0.058	60.0

Table 28. Effect of Aristam prepared with *K. pinnatum* on formalin induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0th day	3rd day		
Control	0.194 ± 0.15	0.363 ± 0.09	0.136	--
Dichlofenac	0.212 ± 0.28	0.269 ± 0.18	0.057	58.02
K. pinnatum 200 µl/kg b.wt	0.21 ±0.019	0.280 ± 0.16	0.070	48.56
K. pinnatum 400 µl/kg b.wt	0.20 ± 0.17	0.264 ± 0.19	0.064	52.59

Table 29. Effect of Arishtam prepared with *C. travancorica* on carrageenan induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0 hour	3rd hour		
Control	0.218 ± .021	0.363 ± 0.07	0.145	--
Dichlofenac	0.216 ± .016	0.260 ± 0.09	0.044	69.41
C. travancorica 200 µl/kg bwt	0.215 ± 0.25	0.280 ± 0.19	0.065	55.29
C. travancorica 400 µl/kg bwt	0.210 ± 0.17	0.257 ± 0.31	0.047	67.06

Table 30. Effect of Arishtam prepared with *C. travancorica* on formalin induced paw edema in Swiss albino mice

Groups	Paw edema in mm		Increase in thickness	% inhibition
	0th day	3rd day		
Control	0.194 ± 0.15	0.363 ± 0.09	0.136	--
Dichlofenac	0.212 ± 0.28	0.269 ± 0.18	0.057	58.02
C. travancorica 200 µl/kg bwt	0.203 ±0.10	0.280 ± 0.14	0.073	46.71
C. travancorica 400 µl/kg bwt	0.204± 0.24	0.267 ± 0.27	0.063	53.52

3.2. B. d. Estrogen induced keratinization

Arishtams prepared with *S. asoca* and the substitutes *K. pinnatum*, *C. travancorica*, *H. brunonis* and *P. longifolia*, were evaluated for estrogen induced keratinization in rat uterus. Administration of Arishtams was found to be effective in preventing the keratinization of uterus epithelium in Wistar rats. From histopathological observations, estradiol treated animals showed highest thickened keratinized layer on rat uterus. The maximum level of thickness was observed in 16th day (Plate 14) after estradiol treatment. The level of thickness was 35.0 μm, 55.4 μm, 25.4 μm, 28.8 μm and 23.1 μm on 8th, 16th, 24th, 32nd, and 40th day of treatment, respectively.

Arishtam prepared with *S. asoca* showed 30.4 μm (Plate 15) thickened keratinization on rat uterus at 8th day, 22.0 μm thickened at 16th day. The thickness of layer reduced to 20.2 μm at 24th day, 6.8 μm and 5.8 μm at 40th day. As reported earlier it is evidenced that Asokarishtam is effective in preventing keratinization induced by estrogen

Arishtams prepared with *K. pinnatum* showed significant reduction of keratinization. The thickness observed in 8th day treatment was 28.1 μm. The thickness reduced to 27.0 μm at 16th day and 18.1 μm at 24th day. The results show that Arishtam prepared with *K. pinnatum* is significantly effective in preventing keratinization in rat uterus induced by estrogen (Plate 16).

Arishtam prepared with *C. travancorica* showed 41.3 μm at 8th day, 45.5 μm at 16th day, 22.1 μm at 24th day, 19.8 μm at 32nd day and 17.4 μm at 40th day of treatment. Thus *C. travancorica* is also effective in preventing keratinization (Plate 17).

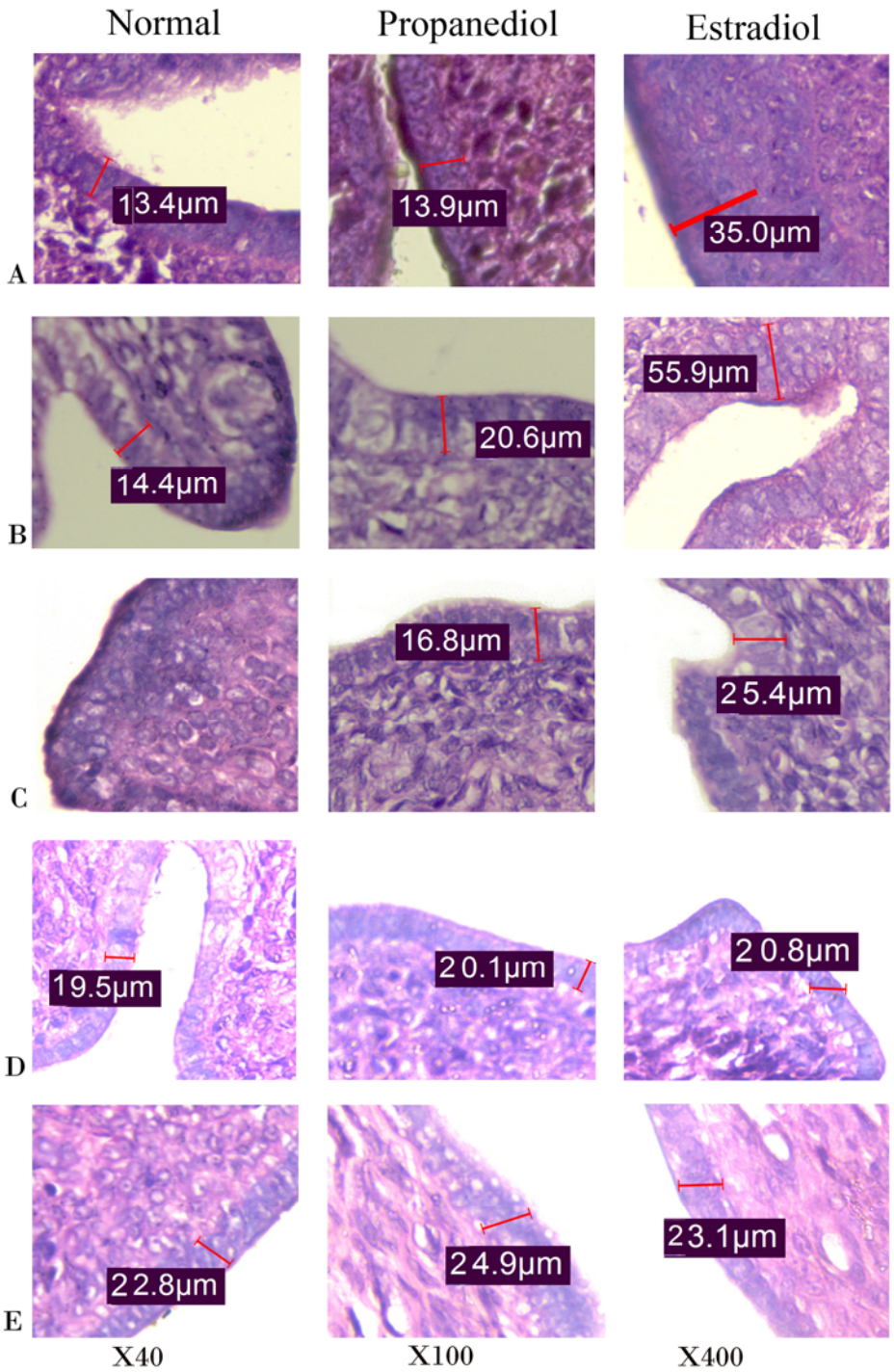
Arishtam prepared with *H. brunonis* showed 49.1μm at 8th day, 35.3 μm at 16th day, 22.4 μm at 24th day, 35.3 μm at 32nd day and 35.5 μm at 40th day of treatment. In this case the thickening of keratinized epithelium was not reduced effectively at the end of the treatment (Plate 18).

Arishtam prepared with *P. longifolia* showed 19.4 μm at 8th day, 25.3 μm at 16th day, 15.1 μm at 24th day, 12.4 μm at 32nd day and 11.8 μm at 40th day of treatment (Plate 19).

In conclusion, the substitute plants except *H. brunonis* were much effective as much as *S. asoca* in reducing estradiol induced keratinization in rat uterus which point out their protective role as a uterine tonic (Table 31). The data generated from the study have provided biological basis for the use of these plants as substitutes of *S. asoca* in therapeutic preparations.

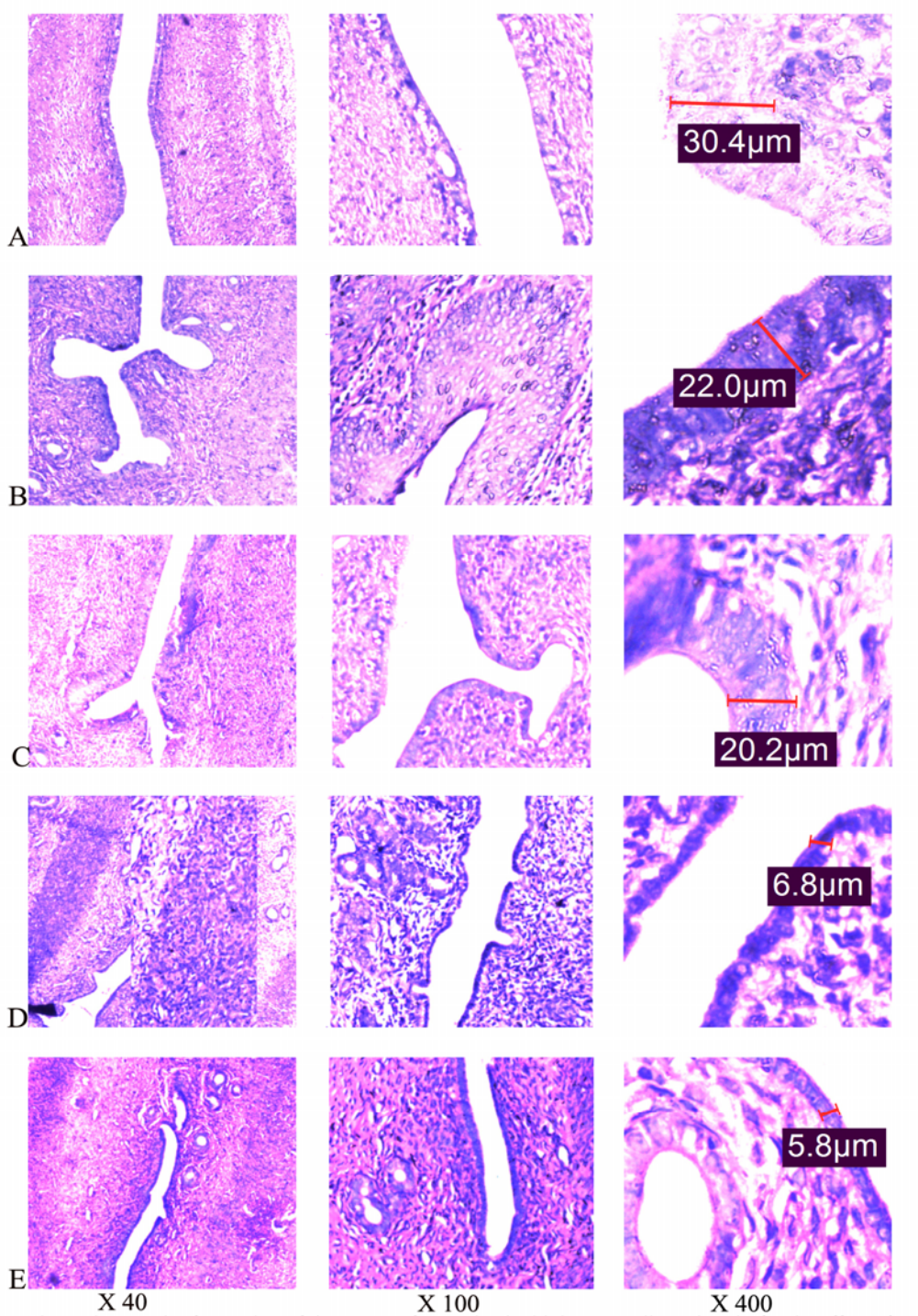
Table 31. Measurement of thickening of keratinized layer in rat uterus induced by estrogen (Thickness expressed in μm)

Plants	8 days	16 days	24 days	32 days	40 days
Normal	13.4	14.4	20.0	19.5	22.8
Propanediol *(vehicle control)*	13.9	20.6	16.8	20.1	20.1
Estradiol	35.0	55.9	25.4	20.8	23.1
S. asoca	30.4	22.0	20.2	6.8	5.8
K. pinnatum	28.1	27.0	18.1	11.3	10.2
C. travancorica	41.3	45.5	22.1	19.8	17.4
H. brunonis	49.1	35.3	22.4	35.3	35.5
P. longifolia	19.4	25.3	15.1	12.4	11.8



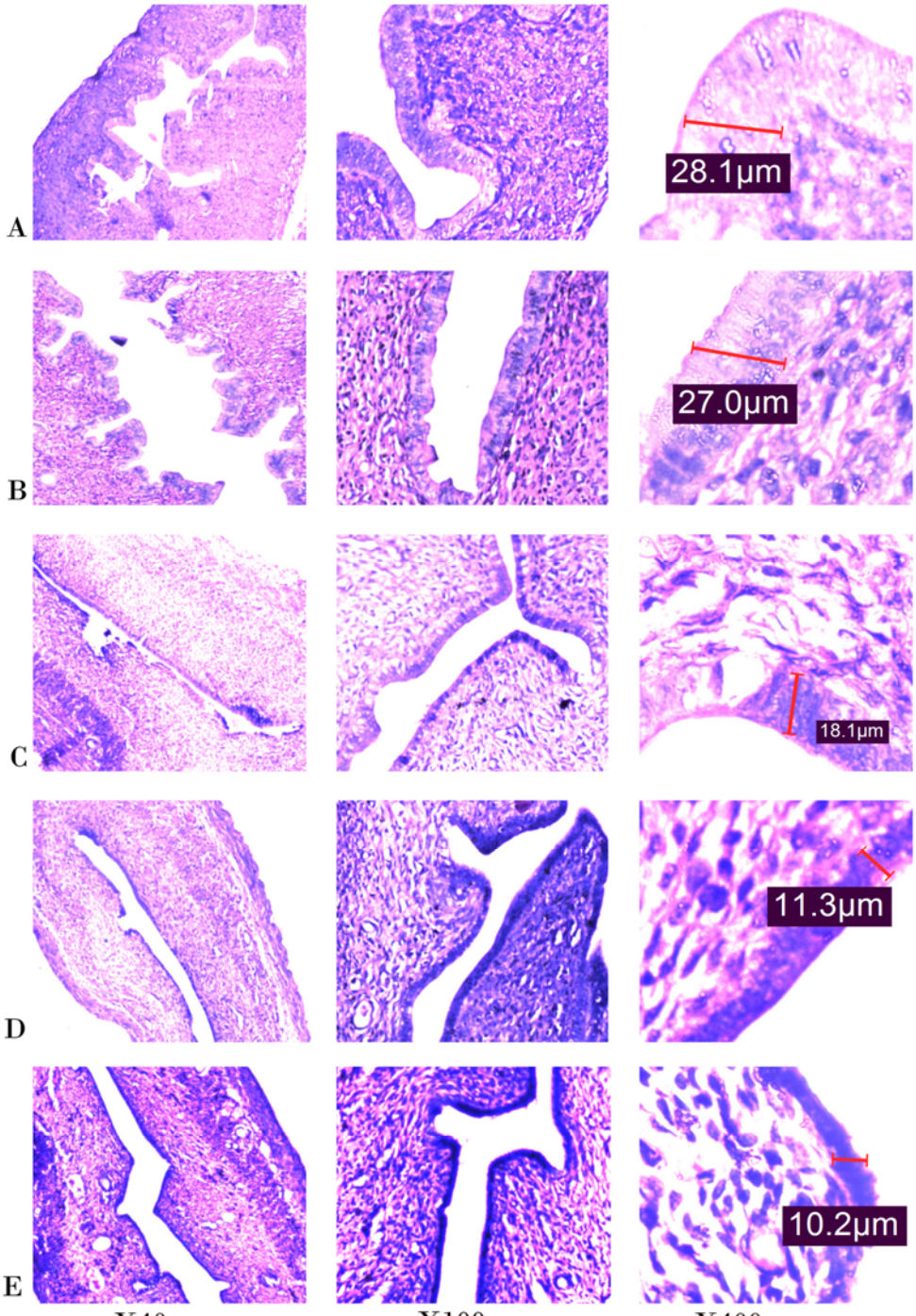
Photomicrograph of a portion of the uterus of normal, propanediol and estradiol treated rats. A. 8th day, B. 16th day, C. 24th day, D. 32nd day and E. 40th day of treatments

PLATE 14



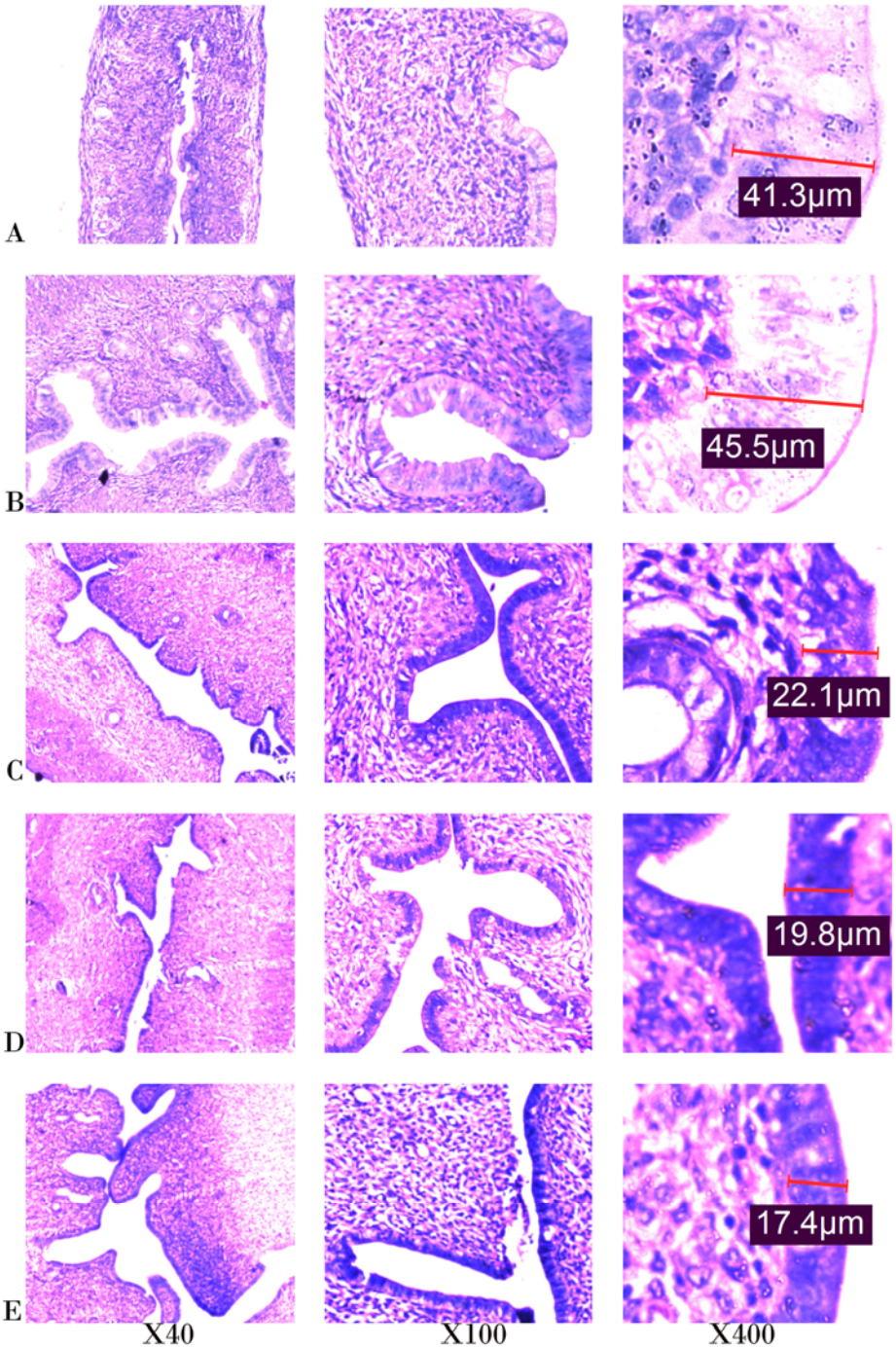
Photomicrograph of a portion of the uterus of rat stained with hematoxylin and eosin for the effect of ayurvedic formulation prepared with *Saraca asoca* on estrogen induced keratinization after 8th day (A), 16 th day (B), 24 th day (C), 32 nd day (D) and 40 th day (E) of treatment.

PLATE 15



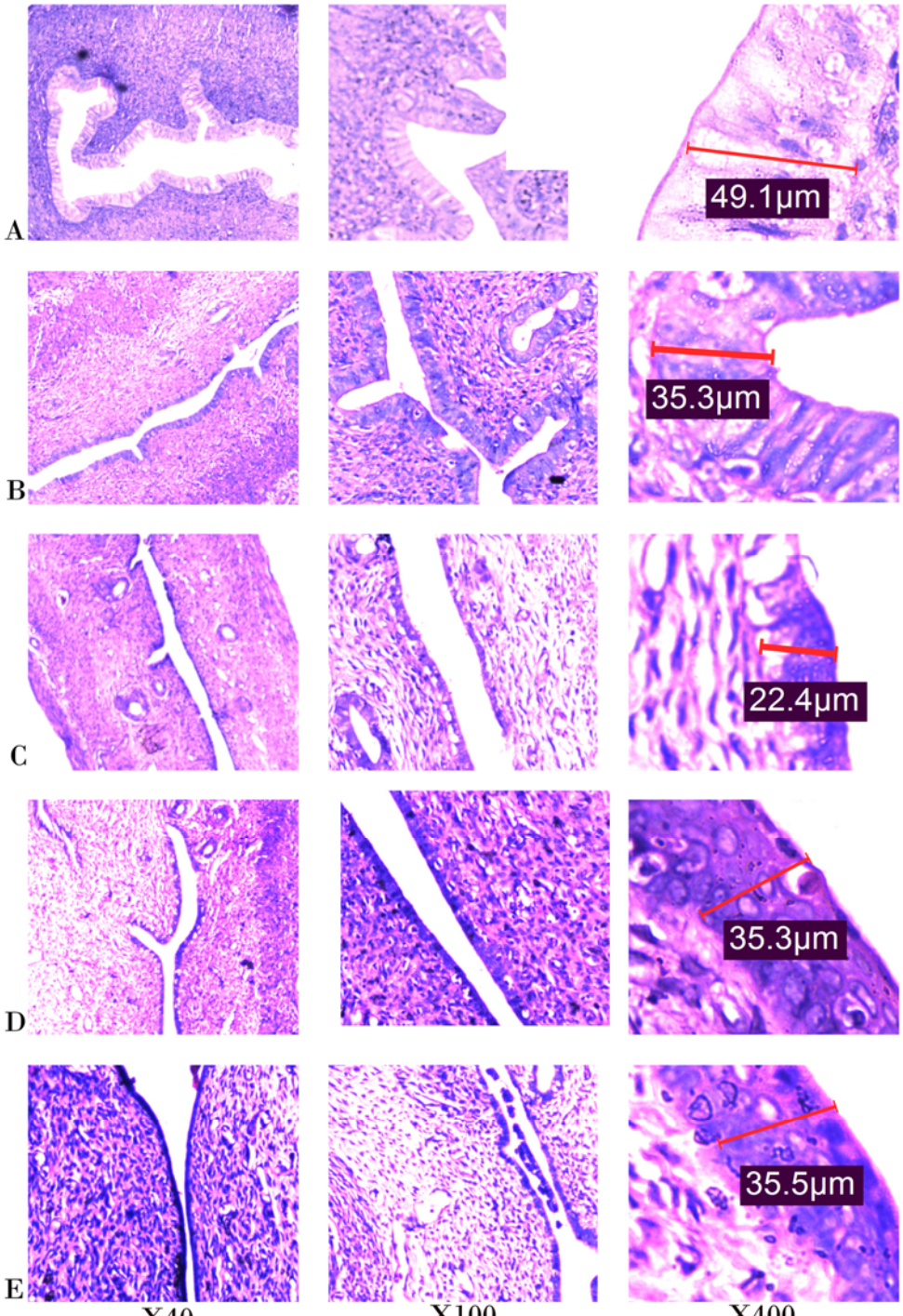
Photomicrograph of a portion of the uterus of rat stained with hematoxylin and eosin for the effect of ayurvedic formulation prepared with *Kingiodendron pinnatum* on estrogen induced keratinization after 8th day (A), 16 th day (B), 24 th day (C), 32 nd day (D) and 40 th day (E) of treatment.

PLATE 16



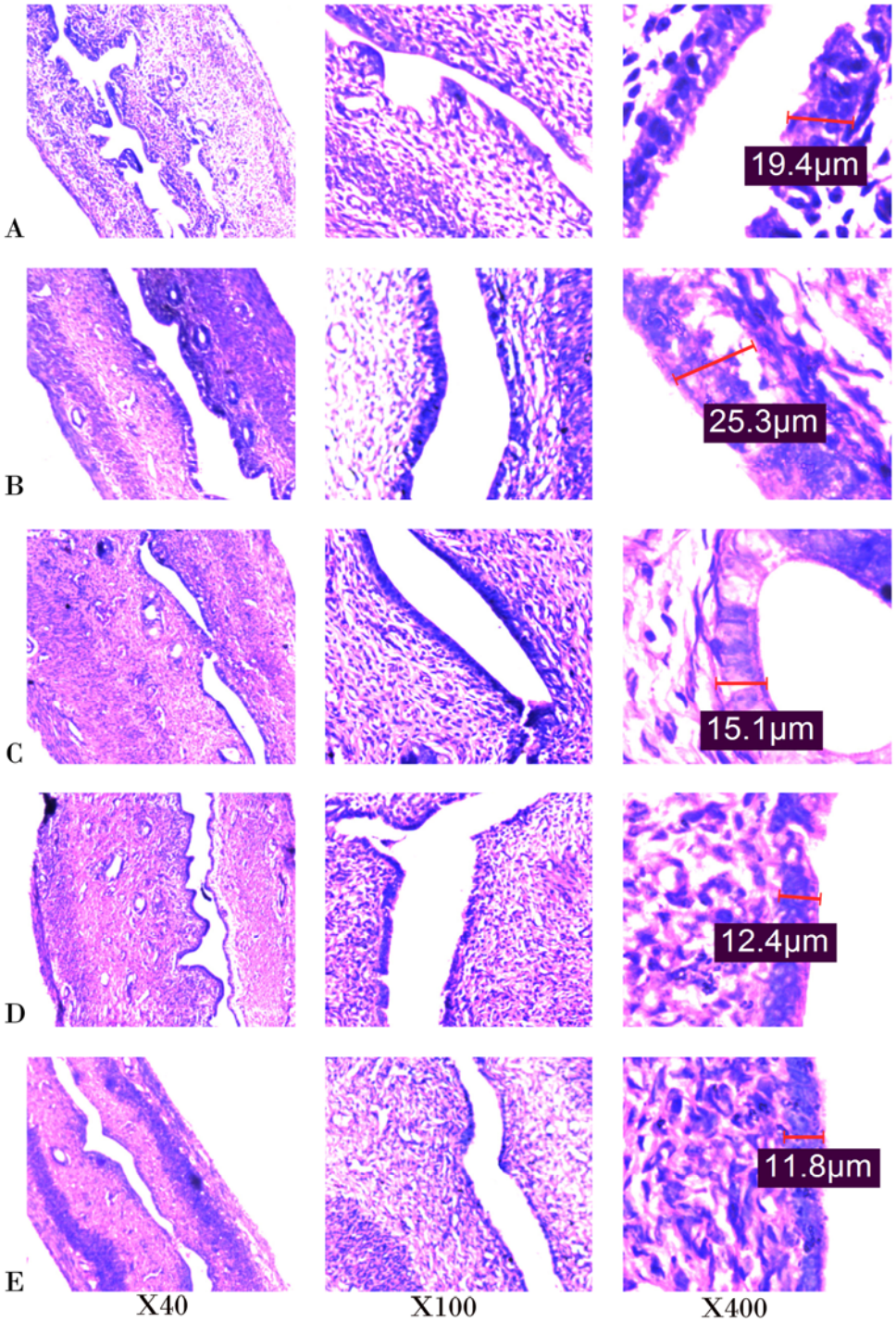
Photomicrograph of a portion of the uterus of rat stained with hematoxylin and eosin for the effect of ayurvedic formulation prepared with *Cynometra travancorica* on estrogen induced keratinization after 8th day (A), 16 th day (B), 24 th day (C), 32 nd day (D) and 40 th day (E) of treatment.

PLATE 17



Photomicrograph of a portion of the uterus of rat stained with hematoxylin and eosin for the effect of ayurvedic formulation prepared with *Humboldtia brunonis* on estrogen induced keratinization after 8th day (A), 16 th day (B), 24 th day (C), 32 nd day (D) and 40 th day (E) of treatment.

PLATE 18



Photomicrograph of a portion of the uterus of rat stained with hematoxylin and eosin for the effect of ayurvedic formulation prepared with *Polyalthia longifolia* on estrogen induced keratinization after 8th day (A), 16 th day (B), 24 th day (C), 32 nd day (D) and 40 th day (E) of treatment.

PLATE 19

3.2.B.e. Effects on antiestrogenic properties

Administration of Arishtam prepared with *S. asoca* and its substitutes *K. pinnatum* and *C. travancorica* was found to be effective in preventing estradiol induced estrogen level (256 pg/ml) in Wistar rats. The level was highly reduced in the Arishtam with *C. travancorica* treated group at the level of 85.63 pg/ml. In the Arishtam with *K. pinnatum* and *S. asoca* showed the reduction of estrogen activity at the level of 124.6 and 154.6 pg/ml (Table 32).

Table 32. Anti-estrogenic activity

Treatment group	Estrogen level (pg/ml)
Normal (without any treatment)	55.88
Estrogen treated (20 μg/animal)	256.5
S. asoca + estrogen (400 μg/animal)	154.6
K. pinnatum + estrogen (400 μg/animal)	124.6
C. travancorica + estrogen (400 μg/animal)	85.63

3.2. B.f. Cyclooxygenase (COX-2)

Western blot analysis demonstrated that uterus and macrophages of LPS treated rats contained detectable levels of COX-2 while it was not present in other parts like serum and intestine. As shown in Figure 19, activity of COX-2 enzyme was high in LPS treated rat and upon administration of Arishtam with *S. asoca* and *K. pinnatum*, the activity was diminished. Less inhibition of Cyclooxygenase activity was detected in the uterus of rat treated with *C. travancorica* and *P. longifolia* arishtams.

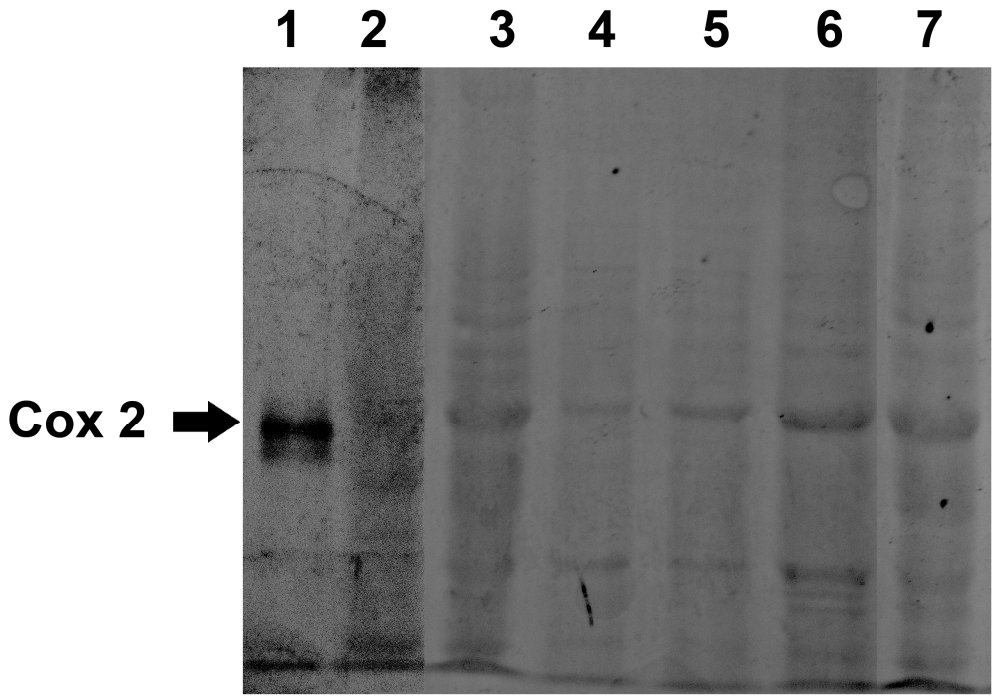


Figure 19. The separated protein bands of uterus were electroblotted to PVDF membrane blocked with buffer containing fat free milk powder for 1 hour and washed. Membrane was incubated with anti-COX-2 antibody (1:5000) for 1 hour (Alpha Diagnostics, USA) washed the membrane and incubated with HRP-conjugated anti-rabbit secondary antibody (1:5000) for 1 hour and developed the membrane using DAB-substrate (Banglore GeNei). Lane 1, Std Cox 2 enzyme; Lane 2, Normal rat; Lane 3, LPS treated; Lane 4, LPS and *S. asoca;* Lane 5, treated arishtam with *K .pinnatum*; Lane 6, treated arishtam with *C. travancorica* and Lane 7, treated arishtam with *P. longifolia*

4. DISCUSSION AND CONCLUSION

The bark of *Saraca asoca* is the principal raw drug in 'Asokaristam' and several other medicinal preparations used in the treatment of gynecological disorders, especially menorrhagia. Tannins contained in the bark provide the main astringent action for halting excessive menstrual bleeding and the mechanism is suggested to be the inhibitory activity on prostaglandin H_2 synthetase (Middelkoop and Labadie, 1985). Due to its extensive use against gynecological disorders, the natural population of *Saraca asoca* has been dwindling over the years in the country. The scarcity of *Saraca asoca* has led to substitution with the bark of other related or unrelated trees. The drug is widely adulterated or substituted with the bark of *Polyalthia longifolia*. Occasionally, *Kingiodendron pinnatum* and *Cynometra travancorica* were also found substituted. The medicinal properties of these species are not well known. Thus, the primary focus of the present study was to evaluate the suitability of the alternatives by assessing their phytochemical constituents and biological properties in comparison with *Saraca asoca*. The biological properties of 'Asokarishtam' prepared with the preferred species, *Saraca asoca* as well as substitutes were also evaluated.

In the comparative phytochemical analysis, the general compounds profiles of *K. pinnatum* and *C. travancorica,* generated through TLC and HPTLC procedures were found to be more or less similar to those of *S. asoca*.

Various reports suggest that the cornification of uterus have a strong and persistent link with chronic inflammation. Although, inflammation is an essential response to injury or infection, chronic inflammation is harmful and causes tissue damage. Inflammation promotes the production of free radicals, which is a contributing factor to the onset of most of the degenerative diseases, including hemorrhagia. In the present study, antioxidant as well as anti-inflammatory activities of *S. asoca* and the

substitute plants were assessed. The results indicate that the bark extracts of *S. asoca* and its substitutes except *H. vahliana* show significant antioxidant properties. Among the substitutes, *K. pinnatum* (acetone as well as methanolic fractions) is found to be most effective in scavenging superoxide radicals; *H. brunonis* (methanolic fraction) effectively inhibits hydroxyl radicals and *C. travancorica* (methanolic fraction) reduces lipid peroxidation. In the anti-inflammatory study, methanolic fractions of the bark of *K. pinnatum*; *C. beddomei, H. brunonis;* bark and leaf extracts of *C. travancorica* show significant activity on acute as well as chronic paw edema in mouse limb.

In the study, *K. pinnatum* is found effective to the same extent as *S. asoca* in reducing estradiol induced keratinization in rat uterus. The elevated level of estrogen in the estradiol administered animals also got significantly reduced by the bark extracts of the substitute plants and the maximum reduction is observed in the case of *C. travancorica*.

Thus, the present study shows that *K. pinnatum* and *C. travancorica* possess phyto-constituents and biological properties similar to *S. asoca*. With respect to antioxidant and anti-inflammatory activities, *K. pinnatum* is closely similar to *S. asoca*. The biological efficacy such as anti-oxidant, anti-keratinization and anti-estrogenic activities of *K. pinnatum* and *C. travancorica* were more or less similar to that of *S. asoca*. However, *C. travancorica* has a pronounced effect on estrogen level and in turn on the estrogen mediated physiological changes in the body. The biological property assays suggest that *K. pinnatum* can be an effective substitute for *S. asoca*. Though acute toxicity was observed in animals administered with *K. pinnatum* extract, no such toxicity was observed when animals were given the Arishtam prepared with it.

The cytotoxic and anticancer properties of *S. asoca* in experimental animals have been reported earlier (Varghese *et al.*, 1993). Present study showed the potent cytotoxic activity of the bark extract of *K. pinnatum*

against DLA and EAC cancer cell lines. The oral administration of the extract significantly increased the life span of ascites tumour bearing mice. This is yet another instance where *K. pinnatum* shows its close similarity with *S. asoca* in biological activities.

Arishtam, a decoction is prepared through fermentation of medicinal plants. In Ayurveda, Arishtams are generally prescribed for managing '*thridosha*' (*Vata, Pitha* and *Kapha*). Ashokarishtam is such a preparation with *S. asoca* as the principal component and other 14 minor constituents. Arishtams were prepared with *S. asoca* and substitutes namely *K. pinnatum*, *C. travancorica*, *H. brunonis* and *P. longifolia* as per Indian Ayurvedic Pharmacopoeia. The prepared Arishtams were individually tested for their anti-oxidant, anti-inflammatory, anti-estrogenic and anti-keratinizing properties.

In the *in vitro* studies, all the prepared Arishtams were found to be effective in scavenging free radicals and inhibiting membrane lipid peroxidation. *In vivo*, the antioxidant enzyme activities were also enhanced in the blood and liver tissues of mice after administration of all the prepared Arishtams. Even though the crude extract of *C. travancorica* bark was less effective in *in vitro* studies, the effect of the Arishtam prepared with it was significantly high compared to *S. asoca* as well as the other substitutes. Among the antioxidant enzymes, SOD catalase and GPX activity got elevated with the administration of Arishtams. An increase in tissue GSH level was also noticed. These findings reveal that the Arishtams may have a pro-oxidant effect that might enhance the total antioxidant system to achieve complete homeostatic redox potential. In the carrageenan and formalin induced paw oedema formation, all the prepared Arishtams showed almost similar percentage of reduction. However, the efficacy of *C. travancorica* was comparatively higher.

In the estradiol induced keratinization analysis on young rats, all the Arishtams except *H. brunonis* showed significant anti-keratinizing effect.

Among the Arishtams prepared with substitutes, *K. pinnatum* was the most effective. Similar results were obtained when individual the crude extract was used. The *P. longifolia* which is widely used for the preparation of Ashokarishtam, was found to differ from *S. asoca* with respect to phytochemical as well as general biological properties. Though the Arishtam prepared with *P. longifolia* showed considerable anti-keratinization property, its efficacy was low compared to the other substitutes evaluated. In all the parameters tested, the effectiveness of individual extract of *K. pinnatum* as well as Arishtam prepared with it is much similar to *S. asoca*. However, with respect to certain parameters *C. travancorica* is found to be more effective.

The anti-keratinization study was performed with 7-day old young rats at their uterine developmental stage. However, recent literature suggests the use of hypothalactomic rat model. Hence for better evaluation of anti-keratinization effect, study needs to be performed with hypothalactomic rats. A well crafted clinical study using Arishtams may provide more confirmation on the use of these medicines in humans. Further, the antiestrogenic effect observed with the Arishtam indicates its potential as a preventive agent in the onset and recurrence of estrogen sensitive breast cancer (Ali and Coombes, 2000). Among the breast cancers reported, majority cases are estrogen dependent and in such patients high level of estrogen in the blood is reported. An accepted chemotherapeutic regime is directed towards keeping normal or below normal level of estrogen in the body so as to achieve reduced tumor growth and prevention of recurrence. In the present study, an increased level of estrogen in the blood in rats induced by exogenously administered estradiol has brought to near normal by the *K. pinnatum* extract. The mechanism for the estrogen reductive property has not yet been studied. However, this result suggests a strong possibility for the extracts in the chemotherapy for breast cancer. Moreover, the anti-oxidant, anti-inflammatory, cytotoxic and anti-tumour efficacy of *K.*

pinnatum observed in the present study is enough to propose its anticancer effect. A mechanistic study in this direction will be a significant contribution in the drug development for breast cancer as well as other estrogen sensitive degenerative diseases.

For testing the COX-2 inhibitory activity of *S. asoca*, the present study was designed to detect a comparative effect of Arishtams prepared with it as well as the substitutes. Lipopolysaccharide (LPS), a cell wall component of the Gram-negative bacteria was administered for the induction of COX-2 enzyme in mice. The results obtained by the Western blot analysis suggest that the LPS induced activity of COX -2 enzyme was diminished on administration of Arishtams with *S. asoca* and *K. pinnatum*. However, less inhibition of cyclooxygenase activity was detected in the case of *C. travancorica* and *P. longifolia* Arishtams treated rat uterus. This result shows that Aristam with *K. pinnatum* possess COX-2 inhibiting activity similar to *S. asoca*.

The data generated from the study thus provide scientific basis for the use of substitute plants in the preparations of Asokarishtam. Though, promising results were obtained with *K. pinnatum* and *C. travancorica*, both these trees are listed under the threatened category, hence the exploitation from the wild source is not advisable. The growth of *K. pinnatum* is much faster than *S. asoca.* It may attain the height of 35 m up to 5 m girth at breast height. Therefore, it is necessary to take up studies on the cultivation aspects of *K. pinnatum* to enhance the availability.

In the phytochemical analysis, the general compound profiles of *Lagenandra ovata* and *L. ovata* and *C. toxicaria,* generated through TLC did not produce any comparative profile with *Kaempferia rotunda*. In the biological studies also no significant antioxidant or anti-inflammatory property was observed with the substitutes. Therefore, further work with the substitutes was not attempted. The results of the phytochemical and biological studies suggest that *Lagenandra ovata* and *L. toxicaria* cannot be substituted for *Kaempferia rotunda*.

5. REFERENCES

Aebi M (1997). Catalase estimation. In: Berg Meyer H V ed., *Methods of enzymatic analysis* 673-684.

Ali S and Coombes RC (2000). Estrogen receptor alpha in human breast cancer: occurrence and significance. *Journal of Mammary Gland Biology and Neoplasia*, 5**:**271-281.

Alzoreky N and Nakahara N (2001). Antioxidant activity of edible Yemeni plants evaluated by Ferryl myoglobin/ABTS assay. *Food Science and Technology Research*, 7, 144.

Alzoreky N, Nakahara N. (2001). Antioxidant activity of edible Yemeni plants evaluated by Ferryl myoglobin/ABTS assay. *Food Science and Technology Research*, 7, 144.

Anjan B N, Hombe G H C and Vasudeva R (2004). A note on air layering in *Saraca asoca* (Roxb). De Wilde. *Journal of Non-Timber Forest Products* 11, 34-35.

Benzie I F F and Strain J J (1999). Ferric reducing/antioxidant power assay: Direct measure of the total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth Enzymol* 299:15-27

Biswas T K and Debnath P K (1972). Asoka (*Saraca indica* Linn)-a cultural and scientific evaluation. *Indian Journal History of Science* 7, 99-114.

Chopra R N (1982). Indigenous Drugs of India, Academic Publishers, Calcutta.

Cordial R R, Baxa-Daguplo B M, Fermanes PM S, Garcia A S, Clavel R M M, Ombac-Herradura M,. Javier J C and Santos R R (2006). Estrogenic activity of *Pueraria phaseoloides* roxb. benth evaluated in ovariectomized rats. *Philippine Journal of Science135* (1) 39-48.

Deepti B, Thaakur S, Babu P S, Priyatamnadh T and Narendra B L (2011). *Saraca asoca* – the management of pain. *Pharmacologyonline* 3, 1039-1045.

Di Rosa M, Giroud J P and Willoughby D A (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenin and turpentine. *Journal of Patholgy* 104, 15.

Dubey N K, Rajesh K and Pramila T (2004). Global promotion of herbal medicine: India's opportunity. *Current Science* 86, 37-41

Elizabeth K and Rao MNA (1990). Oxygen radical scavenging activity of Curcumin. *Int J Pharm*, 58, 237-240.

Gadgil M and Guha R (1992). This Fissured Land. Oxford University Press, Delhi, 485-514.

Haeuber R (1993). Development and deforestation: Indian forestry in perspective. *Journal of Developing Areas* 27(4), 485-514.

Hafeman D G, Sundae R A and Houestra W G (1974). Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. *Journal of nutrition* 104, 580-587.

Hattori M, Nakabayashi T, Lim Y A, Miyashiro H, Kurokawa M, Shiraki K, Gupta M P, Correa M and Pilapitiya U (1995). Inhibitory effects of various Ayurvedic and Panamanian medicinal plants on the infection of herpes simplex virus 1, *in vitro and in vivo. Phytotherapy Research* 9, 270-276.

Ialenti A, Ianaro A, Moncada S, Di Rosa M (1995). Modulation of acute inflammation by endogenous nitric oxide. *Eur. J.Pharmacol.* 211:177-184.

Klivenyi P, Kiaei M, Gardian G, Calingasan N Y and Beal M F (2004). Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis *Journal of Neurochemistry* 88, 576–582.

Kumar A, Gladson M, Ahmad N and Tyagi K (2002). Oestrogenic effects of an ayurvedic polyherbal formulation, ashokarishta. *Singapore Journal of Obstetrics and Gynaecology* 33(1): 14-22.

Kuraoka I, Robins P, Masutani C, Hanaoka F, Gasparutto D, Cadet J, Wood R D and Lindah T (2001). Oxygen Free Radical Damage to DNA. *The Journal of Biological Chemistry* 276, 49283–49288.

Logani MK and Davis RE (1979). Lipid peroxidation in biologic effects and antioxidants. *A Rev Lipids*;15:485-93.

Mc Cord J M and Fridovich I (1969). Superoxide dismutase enzyme function for erythrocaprein. *JB*, 224, 6049.

Middelkoop T B, Labadie R P (1985). The action of *Saraca asoca* Roxb. de Wilde bark on the PGH2 synthetase enzyme complex of the sheep vesicular gland. *Zeitschrift für Naturforschung* C 40, 523-526

Mitchell R N and Cotran R S (2000). Robinsons Basic Pathology, Ed 7. Harcourt Pvt. Ltd., New Delhi, India, 33-42.

Moron M A, Depierre J W, Manner and Vick B (1979). Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat liver. *Biochimica et Biophysica Acta* 582, 67-68.

Nergard CS, Diallo D, Inngjerdingen K, Michaelsen TE, Matsumoto T, Kiyohara H, Yamada H, Paulsen BS (2005). Medicinal use of *Cochlospermum tinctorium* in Mali Anti-ulcer- radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. *Journal of Ethnopharmacology* 96, 255–269.

Ohkawa H, Ohishi W, Yagi K. (1979). Assay for lipid peroxides in animal tissues by thio barbituric acid reaction. *Biochemistry*, 95, 351-358.

Parhizkar S, Latiff L A, Rahman S A, Ibrahim R, Mohammad and Dollah A (2011). *In vivo* estrogenic activity of *Nigella sativa* different extracts using vaginal cornification assay. *Journal of Medicinal Plants Research* 5(32), 6939-6945.

Patwardhan B (2000). Ayurveda: The designer medicine. *Indian Drugs* 37, 213-227.

Patwardhan B (2000). Ayurveda: The designer medicine. *Indian Drugs* 37, 213-227.

Pham-Marcou T, Beloeil H, Sun X, Gentili M, Yaici D, Benoit G, Benhamou D and Mazoit J (2008). Antinociceptive effect of resveratrol in carrageenan-evoked hyperalgesia in rats: Prolonged effect related to COX-2 expression impairment. *Pain* 140, 274–283.

Pokorny J, Yanishlieva N V and Gordon M H (2001), *Antioxidants in food*, Boca Raton: CRC press, 324–344.

Poornima B (2010), Adultration and substitution in herbal drugs a critical analysis. *International Journal Of Research In Ayurveda & Pharmacy* 1 (1), 8-12

Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, Verma R and Bajpai A (2009). Saraca asoca (Ashoka): *A Review Journal of Chemical and Pharmaceutical Research* 1 (1), 62-71.

Pulok M K (2002). Quality Control of Herbal drugs, Business Horizons, New Delhi. (ed) 113-117

Rajshekharan P E (2002). Herbal Medicine. In World of Science, *Employment News* 21-27, 3.

Ravikumar R and Ved D K (2000). 100 Red Listed Medicinal Plants of South India. FRLHT, Bangalore 334-336

Sarwar G (2002). The phytochemical and phytopharmacological studies on *Saraca ndica, Capparis deciduas* and *Lotus gracinii* P hD Thesis, Department of Pharmacognosy/University of Karachi

Sasidharan N, Muraleedharan P K (2000). Survey on the commercial exploitation and consumption of medicinal plants by the drug industry in Northern Kerala. *KFRI Research Report* No. 193.

Sasmal S, Majumdar S, Gupta M, Mukherjee A and Mukherjee P K (2012). Pharmacognostical, phytochemical and pharmacological evaluation for the antipyretic effect of the seeds of Saraca asoca Roxb. *Asian Pacific Journal of Tropical Biomedicine* 1-2.

Satyavati G V, Prasad D N, Sen S P and Das P K (1970). Further studies on the uterine activity of *Saraca indica* Linn. *Indian J. Med. Res.* 58: 947-960.

Sharma P C, Yelne M B, Dennis T J (2005). Database on medicinal Plants used in Ayurveda. New Delhi: Central Council for Research in Ayurveda and Siddha, Department of ISM & H, Ministry of Health and Family Welfare (Govt. of India) 3, 76-87.

Turner D C, Bagnara J T (1971). General Endocrinology, 5th Edn. Tokyo: W B Saunders Co. 516.

Urasopon N, Hamada Y, Asaoka K and Poungmali U and Malaivijitnond S (2008). Isoflavone content of rodent diets and its estrogenic effect on vaginal cornification in *Pueraria mirifica*-treated rats. *Science Asia* 34, 371–376.

Varghese C D, Nair C S, Beena P and Panikkar K R (1993). Effect of Asoka on the intercellular glutathione levels and skin tumour promotion in mice. *Cancer Letters* 69.45 -50.

Velioglu YS, Mazza G, Gao L and Oomah B D (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural Food Chemistry* 46, 4113 -4117.

Vijayasaradhi S and Gupta P D (1988). Keratinization of rat vaginal epithelium II. Immunofluorescence study on keratin filaments in cycling and estrogen primed rats. *Journal of Biosciences* 13, 2 109–116.

Warrier P K, Nambiar V P, Ramankutty C (1995). In: Indian Medicinal Plants: A Compendium of 500 Species Viol 4, Ed. Orient Longman, Hyderabad, 366-70.

Wong C, Li H, Cheng K, Chen F (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry* 97, 705–711.

World Health Organization [WHO] (2000). General guidelines for methodologies on research and evolution of traditional medicine. *Geneva: WHO*.