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Research Article

**EXTRACTION OF PHYTOCONSTITUENTS AND
EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF
AERIAL PARTS OF SARCOSTIGMA KLEINII WIGHT & ARN**Visal C. S^{1*}, ²Dr. Prasobh G.R., ³Dr. Sandhya S.M., ⁴Nishad V. M., ⁵Anu S.¹Sreekrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram Dist, Kerala.**Article Received:** August 2019**Accepted:** September 2019**Published:** October 2019**Abstract:**

According to World Health Organization, 80% of the people living in the rural areas depend on medicinal plants as primary health care system. These practices are solely based on the knowledge of traditional use of medicinal plants; but traditional use of medicinal plants is continuously decreasing with the easy availability of the modern medicines and unavailability of information of local flora. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Here we will be extracting phytoconstituents from aerial parts of Sarcostigma Kleinii and its phytochemical screening will be performed and its antioxidant activity is also evaluated

Keywords: Sarcostigma Kleinii , Antioxidant activity.**Corresponding author:****Visal C.S,**

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INTRODUCTION:

Plants have great economic and medicinal importance throughout the world. The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated as 2, 50,000, of these, only about 50-60% have been screened for biological activity. [1] In recent times, medicinal plants occupy an important position for being the paramount sources of drug discovery, irrespective of its categorized groups herb, shrub or tree. Plants have been indispensable in treating diverse forms of disease. [2]

According to World Health Organization, 80% of the people living in the rural areas depend on medicinal plants as primary health care system. These practices are solely based on the knowledge of traditional use of medicinal plants; but traditional use of medicinal plants is continuously decreasing with the easy availability of the modern medicines and unavailability of information of local flora of medicinal importance. Many plants are widely used by all section of the community, whether directly as folk remedies or the medicaments of the different indigenous system as well as in modern medicine. Furthermore, about 74% of all plant-derived drugs in worldwide clinical uses have been discovered through follow-up investigation of the ethno medicinal uses of plants. Therefore, it is essential for drug discovery to record and preserve the traditional knowledge of medicinal plants that mostly depends on local practitioners and field surveys. [3]

India has one of the richest plant medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India and there are over 1.5 million practitioners of traditional medicinal system using medicinal plants in preventive, promotional and curative applications. In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. [4]

The market for ayurvedic medicines is estimated to be expanding at 20% annually. Sales of medicinal plants have grown by nearly 25% in India in past ten years; the highest rate of growth in the world. But the per capita expenditure in India on medicines per

annum is amongst the lowest in the world. In other developing countries too, plants are the main source of medicine. Two of the largest users of medicinal plants are China and India. Traditional Chinese Medicine uses over 5000 plant species; India uses about 7000. Recently even developed countries, are using medicinal systems that involve the use of herbal drugs and remedies. Undoubtedly the demand for plant-derived products has increased worldwide.⁵ The demand is estimated to grow in the years to come fuelled by the growth of sales of herbal supplements and remedies. This means that scientists, doctors and pharmaceutical companies will be looking at countries like China, India, etc. for their requirements, as they have the most number of medicinal plant species.

Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine, camptothecin and drugs like Cinchona (antimalarial), Datura (mydriatic), Liquorice (demulcent and expectorant), Nutmeg (carminative, digestive), Rauwolfia (antihypertensive), Senna (laxative) etc are widely used as herbal medicines. [6]

The medicinal properties of plants are due to vital chemicals present in them and these chemicals are called phytochemicals. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids, coumarins, flavanoids etc. Phytochemicals literally mean "plant chemicals." Scientists Have identified thousands of different phytochemicals, found in vegetables, fruits, beans, whole grains, nuts and seeds. Eating Lots of plant foods rich in phytochemicals may help to prevent at least one in every five cases of cancer, as well as other serious ailments such as heart disease. [7]

Phytochemicals are active metabolites that necessarily require extraction and isolation from their natural sources with many unwanted materials. The phytochemicals can come singly or as a mixture of important substances to form active principle responsible for its activity. There are now more rapid strategies for chemical characterizations of phytoconstituents of natural products as well as assessing the bioactivities of the natural products; Coupled or hyphenated methods of separations, isolations, purifications and characterizations are now very appropriate. These include LC/UV, LC/MS, LC-FTIR, LC-NMR, LC/UV-DAD, MS/MS, LC-MS/MS, Q-TOF-MS, CE- capillary electrophoresis,

with its added advantage of use of very little solvent consumption, lower costs, short time of analysis and its generally economical; MECC- micellar electrokinetic capillary chromatography, this is when the capillary electrophoresis is in conjunction with electrochemical detections usually along with assay experiments; HSCCC-high speed counter-current chromatography; SPME- solid phase micro extraction; SCFEC- supercritical fluid extraction chromatography; ESI; HPLC- MS/ESI. Introduction of FT [fourier transform] in structural elucidations have increased the enormous power of spectroscopies like IR and NMR. [8]

Medicinal plants as potential source of therapeutic aid has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

ICACINACEAE:

The Icacinaeae are a family of flowering plants, consisting of trees, shrubs, and lianas, primarily of the tropics. The family was traditionally circumscribed quite broadly, with around 55 genera totalling over 400 species. In 2001, though, this circumscription was found to be polyphyletic, and the family was split into four families in three different orders: Icacinaeae sensu stricto Pennantiaceae (Apiales), Stemonuraceae (Aquifoliales) and Cardiopteridaceae (also Aquifoliales). Icacinaeae sensu stricto contains about 150 species, distributed into about 35 genera. [9]

Icacina senegalensis extracts have shown activity against malaria parasites. [10] *Nothapodytes nimmoniana* show antimicrobial activity and are traditionally used as a remedy for malaria and has anti- inflammatory activities. [11] *Rhaphiostylis beninensis* root possess anti- inflammatory activities.

Sarcostigma kleinii belongs to the family Icacinaeae. It is a wild edible plant traditionally used by the tribes in the Parambikulam Wildlife Sanctuary, Kerala, India. [12] The plant's bark and leaves are bitter, acrid, thermogenic, anthelmintic, digestive, carminative, diuretic, anaphrodisiac, depurative, vulnerary and stomachic. They are useful in vitiated conditions of vata, cephalalgia, gastropathy, anorexia, flatulence, helminthiasis, strangury, indolent ulcers, leprosy, skin diseases,

hysteria and epilepsy. The oil is bitter, anaphrodisiac, anthelmintic, vulnerary and depurative. It is useful in vitiated conditions of vata, helminthiasis, foul ulcers, and leprosy and skin diseases.¹³

EXTRACTION:

Extraction is first pre-purification step in the isolation and characterization of active compound(s) of a medicinal plant. The type of solvent for extraction may range from non-polar to polar solvent depending on the targeted class of bioactive component(s). Though the method is relatively simple, some of the drawbacks include: long extraction time, labour intensity, high solvent consumption and inadequate reproducibility. In traditional medicine practice, ethanol and water are the most widely used solvents. The bioactive components of medicinal plants are usually unknown, and the nature of the solvent used affects the composition of the crude extract. Therefore, solvents such as hexane, dichloromethane, ethyl acetate, acetone, methanol, propanol, water or a combination of solvents are used in laboratory settings. Acetone has been adjudged to be the best solvent of plant extract for bioassay because it extracts a broad spectrum of components (polar and non-polar), is miscible with all other solvents, is highly volatile, and exhibit low toxicity to biological organisms in various assays.

Temperature is also an important factor in extraction, drying and storage of plant extracts because of varying compound stability due to chemical degradation, losses by volatilization and oxidation. Milder extracting and drying temperatures are required to avoid loss of activity by plant extracts possibly due to thermal decomposition. Storage of plant extracts, fractions or isolated pure compounds should be done at 4°C in the dark to avoid any negative influence of temperature and light.

The Soxhlet method is one of the most popular methods for extraction from the natural products. Here the dried powdered plant is continuously extracted in a soxhlet apparatus with a range of solvents, starting in turn with ether, petroleum and chloroform (to separate lipids and terpenoids) and then using alcohol and ethyl acetate (to separate more polar components). However continuous hot soxhlet extraction is not preferable to heat sensitive constituents. [14]

The extract obtained is clarified by filtration and is then concentrated in vacuum, it usually carried out in a rotary evaporator. The concentrated extract may deposit as crystals on standing. If so, these should be collected by filtration and their homogeneity tested

for by chromatography in several solvents. If a single substance is present, the crystals can be purified by recrystallisation and the material is available for further analysis. In most cases mixtures of substance

will be present in the crystals and it will then necessary to re dissolve them in suitable solvent and separate the constituent by chromatography.

Plant Profile

Sarcostigma kleinii belongs to the family Icacinaceae and it is a woody climber.



Fig:1 *Sarcostigma kleinii*

BOTANICAL INFORMATION:

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Celastrales
Family	Icacinaceae
Genus	Sarcostigma
Species	<i>Sarcostigma kleinii</i>

SYNONYMS:

English	<i>Sarcostigma kleinii</i>
Hindi	Mukhajali
Konkani, Marathi	Davabindu
Kannada	Puvanna
Tamil	Puvennai, Ota
Malayalam	Vellayodal

DESCRIPTION OF PLANT:

They are Woody climbers, branchlets are glabrous. Leaves are oblong-lanceolate, apex acuminate, base rounded or obtuse; lateral nerves 8 pairs, reticulate; petiole 5-12 cm long. Flowers are 3-6 together, yellow; calyx cupular, 2 mm across, 5-toothed; petals 3-5 mm long, oblong, recurved; female flowers mostly from old wood, ovary 1-celled, densely hairy; stigma sessile, discoid; pistillode in male flowers are conical. Fruits are Drupe, orange-yellow in colour. [23]

MEDICINAL PROPERTY OF THE PLANT:

The plant's bark and leaves are bitter, acrid, thermogenic, anthelmintic, digestive, carminative, diuretic, anaphrodisiac, depurative, vulnerary and stomachic. They are useful in vitiated conditions of vata, cephalalgia, gastropathy, anorexia, flatulence, helminthiasis, strangury, indolent ulcers, leprosy, skin diseases, hysteria and epilepsy. The oil is bitter, anaphrodisiac, anthelmintic, vulnerary and depurative. It is useful in vitiated conditions of vata, helminthiasis, leprosy and skin diseases. [24]

GEOGRAPHICAL DISTRIBUTION:

It is a wild edible plant traditionally used by the tribes in the Parambikulam Wildlife Sanctuary, Kerala. They are usually found in Western Ghats at Low Altitudes. They are distributed widely in Maharashtra: Kolhapur, Karnataka: Chikmagalur, Coorg, Kerala: All districts, Tamil Nadu: Coimbatore, Kanniyakumari, Nilgiri, Tirunelveli.²⁵

METHODOLOGY:**Preparation of plant material:**

Aerial parts of the plant *Sarcostigma kleinii* after collection were washed with water to remove undesirable materials and were dried in shade. The dried plant material was ground to coarse powder. The powdered drug was then subjected to hot extraction using Soxhlet apparatus, as per the standard procedure and the crude extracts were subjected to preliminary phytochemical screening.

Method of extraction-general procedure [26]:**Hot extraction using Soxhlet method:**

The crude drug was exhaustively extracted with a solvent in a soxhlet extractor using the hot continuous percolation method. The extract was concentrated under reduced pressure at bath temperature below 40°C to a syrupy consistency after which it was evaporated to dryness.

Weighed 60g of powdered drug of *Sarcostigma kleinii* and packed in a filter paper thimble and then it was extracted with 300ml of the solvent ethanol

using the hot continuous percolation method not less than 48hr. Then the extract was concentrated and evaporated to dryness.

Preparation of petroleum ether fraction:

The ethanolic extract obtained was then divided in to two portions and triturated with certain amount of petroleum ether and it was filtered; the filtrate obtained was then concentrated evaporated to dryness to obtain petroleum ether fraction of ethanol.

PHYTOCHEMICAL SCREENING [28,29,30,31]:

A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phyto-chemical screening for the following lines.

1) Detection of Alkaloids

The extracts were individually dissolved in dil HCl and filtered

- (a) **Dragendorff's Test:** To 0.5ml of filtrate, added 1ml of Dragendorff's reagent was .An orange red precipitate producing immediately indicates the presence of alkaloids.
- (b) **Mayer's Test:** To 1ml of filtrate, added a few drops of mayer's reagent, Formation of pale precipitate shows the presence of alkaloids.
- (c) **Hager's Test:** To 1ml of the filtrate added few drops of Hager's reagent, formation of yellow precipitate shows the presence of alkaloids..
- (d) **Wagner's Test:** To 1ml of filtrate added few drops of Wagner's reagent, Formation of yellow or brown precipitate will confirm the presence of alkaloids.

2) Detection of Carbohydrates

The extracts were individually dissolved in distilled water and filtered

- (a) **Molisch's Test:** In a test tube containing 2.0ml of filtrate, 2.0 drops of freshly prepared 20% alcoholic solution of α -naphthol was added and mixed. To this solution, added concentrated sulphuric acid through the sides. Formation of violet ring at the junction of the solution indicates the presences of carbohydrates.
- (b) **Fehling's Test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red or brick red precipitate indicates the presence of carbohydrate.
- (c) **Benedict's test:** Extract was treated with Benedict's reagent (copper sulphate

+sodium citrate + sodium carbonate in water), and heated for 10 minutes in water bath. Formation of orange red precipitate indicates the presence of carbohydrate (reducing sugars).

3) Detection of Glycosides

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

(a) **Legal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide pink to blood red colour indicates the presence of glycosides.

4) Detection of Saponin

(a) **Foam Test:** The drug extract or dry powder on vigorous shaking with water gives persistent foam for 10min which is an indication of saponin glycosides.

5) Detection of Phenols

(a) **Ferric Chloride Test:** To 1.0ml of extract, 2.0ml of distilled water followed by drops 10% solution aqueous ferric chloride solution were added. Formation of blue or green indicates the presence of phenols.

(b) **Lead Acetate Test:** To 1.0ml of extract was diluted to 5ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. Formation of yellow precipitate indicates the presence of phenol.

6) Detection of Steroids and Triterpenoids

(a) **Salkowski Reaction:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid shaken and allowed to stand appearance of greenish yellow colour indicates the presence of triterpenoids.

(b) **Libermann- Burchard Reaction:** To the extract, add 2-3 drops of chloroform and a few drops of acetic acid and concentrated sulphuric acid is added along the sides of the test tube, Formation of red ring at the junction of two layers indicates the presence of triterpenoids.

(c) To 2 ml of the extract, added 2 ml chloroform and 2ml Con. H₂SO₄ and shaken well; red colour was produced in the upper layer and a greenish yellow fluorescence produced in the lower layer indicates the presence of steroids.

7) Detection of Tannins

(a) **Ferric Chloride Test:** To 1.0ml to 2.0ml of extract, a few drops of 5%aqueous ferric chloride solution was added. Formation of bluish black colour, which disappears on addition of a few ml of dilute sulphuric acid, and formation of a yellowish brown precipitate, indicates the presence of tannins.

(b) **Lead Acetate Test:** In the test tube containing about 5.0ml of extract, a few drops of 1% solution of lead acetate was added. Formation of yellow or red precipitate indicates the presences of tannins.

8) Detection of Flavonoids

(a) **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution formation of yellow colour and disappearance of yellow colour on addition of dil sulphuric acid indicates the presence of flavonoids.

(b) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution, formation of yellow colour indicates the presence of flavonoids.

9) Detection of fixed oils and fats

(a) **Stain test:** Small quantities of extract were pressed between two filter papers appearance of oil stain indicates presence of fixed oils and fats.

10) Detection of Proteins and Amino Acids

(a) **Biurette Test:** To 3 ml test solution added 4% NaOH and few drops of 1% CuSO₄ solution. Violet or purple colour formed indicates the presence of proteins.

(b) **Ninhydrin Test:** 3 ml of the test solution and 3 drops of 5% ninhydrin solution was heated for 10 min on a boiling water bath. Purple or bluish colour indicates the presence of proteins.

(c) **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid formation of yellow colour indicates the presence of proteins.

ANTIOXIDANT STUDY:

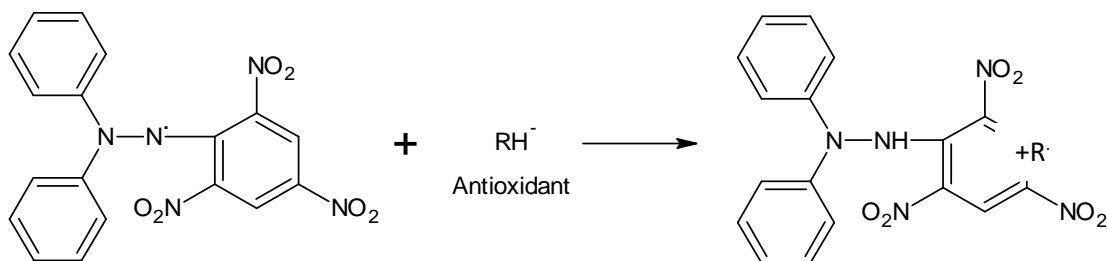
The petroleum ether fraction of ethanolic extract was subjected to antioxidant studies by DPPH method and Nitric oxide scavenging activity.

(a) DPPH METHOD [34]

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). The structure of DPPH and its reduction by an antioxidant are shown below. When Antioxidants react with DPPH (Diphenylpicrylhydrazyl), which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPH-H (Diphenylpicrylhydrazine) and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form results in decolorization (yellow colour) with respect to the number of electrons

captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When a solution of DPPH

is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour.



2, 2-diphenyl-1-picrylhydrazyl (purple)

2, 2-diphenyl-1-picrylhydrazine (colourless)

Requirements:

Ethanol
Diphenylpicrylhydrazyl

Preparation of test and standard solution:

1mg/ml solution of the petroleum ether fraction of extract and the standard in ethanol was prepared and then serially diluted to obtain lower concentration (100-500 μ g/ml)

Reagent preparation:

0.1Mm solution of DPPH was prepared by dissolving 1.95mg of DPPH in ethanol. After dissolving

completely the solution was made up to 50ml with ethanol.

Procedure [35]:

1ml of DPPH in ethanol (0.1Mm) was added to 3.0 ml of extract and standard solutions in ethanol at different concentration. Thirty minutes later, the absorbance was measured at 517nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity; the capability to scavenge the DPPH radical was calculated using the following equation

$$\text{Scavenged (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100 \quad \text{DPPH}$$

The antioxidant activity of extract was expressed in terms of IC₅₀ (the concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

1) NITRIC OXIDE SCAVENGING ACTIVITY [36]

Nitric oxide (NO[•]) has been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO[•], its contribution to oxidative damage is also reported. This is due to the fact that NO[•] can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH[•] and NO[•]. The procedure is based on the principle that, sodium nitro-prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce

nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

Requirements:

Sodium nitroprusside
Phosphate buffer saline pH 7.4
DMSO
Sulfanilic acid reagent
Naphthyl ethylene diamine dihydrochloride

Preparation of test and standard solution:

1mg/ml solution of the petroleum ether fraction of extract and the standard in DMSO was prepared and then serially diluted to obtain lower concentration (100-500 μ g/ml)

Reagent preparation:

Sodium nitro prusside solution was prepared by

dissolving 0.3g in 100 ml distilled water. The nitrate mixture (5 ml) consisting of sodium nitro prusside 4 ml and 1 ml of phosphate buffer saline (PBS) was prepared. sulfanilic acid reagent(0.33g sulfanilic acid dissolved in 20% glacial acetic acid and volume made to 100 ml and the solution is heated until dissolves) was prepared , 0.1% w/v Naphthyl ethylene diamine dihydrochloride was prepared in 50% glacial acetic acid.

Procedure:

0.5ml of nitrate mixture was added to 1ml of extract and standard solutions at different concentrations and incubated at room temperature for 150 min, after incubation 1ml of sulfanilic acid reagent and 0.1% NEDD was added mixed well and allowed to stand at

room temperature for 10 min and then the absorbance was measured at 546 nm.

Percentage inhibition was calculated by using the formula

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}} \times 100$$

RESULTS:

1) PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening of ethanolic and petroleum ether fraction of ethanolic extract were done and it revealed the presence of phytoconstituents as given in the table

Phytochemical screening

Sl no:	Chemical test	Ethanolic extract	Petroleum ether fraction of Ethanolic extract
1	Alkaloids	+	-
2	Carbohydrates	+	-
3	Glycosides	-	-
4	Saponins	-	-
5	Phenols	+	+
6	Triterpenoids	+	+
7	Phytosterols	+	+
8	Tannins	+	-
9	Flavonoids	+	+
10	Fixed oils and fats	-	-
11	Proteins and Aminoacids	-	-

1) ANTIOXIDANT ACTIVITY

The current study involves the estimation of antioxidant property of petroleum ether fraction of ethanolic extract *in vitro*; by the DPPH method and nitric oxide radical scavenging method,

a) DPPH method

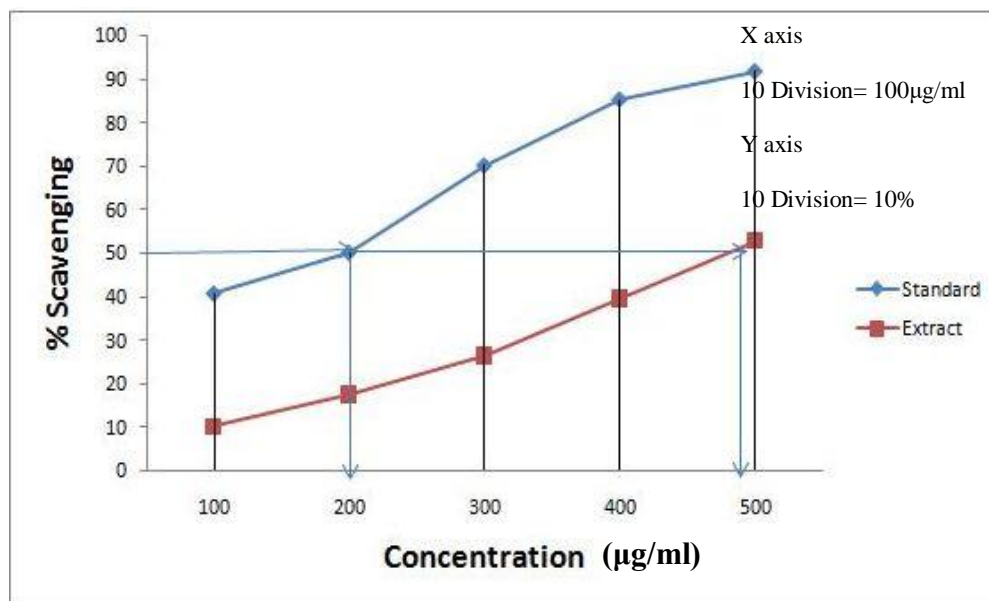
Absorbance of control= 0.984

Table: 1. Scavenging of DPPH radical by standard (Ascorbic acid)

Sl no	Concentration (µg/ml)	Absorbance(nm)	% scavenging
1	100	0.583	40.75
2	200	0.412	50.13
3	300	0.294	70.12
4	400	0.145	85.26
5	500	0.081	91.76

Table: 2. Scavenging of DPPH radical by petroleum ether fraction

Sl no	Concentration ($\mu\text{g/ml}$)	Absorbance (nm)	% scavenging
1	100	0.884	10.16
2	200	0.812	17.47
3	300	0.725	26.32
4	400	0.596	39.43
5	500	0.465	52.74

**Graph 1:** DPPH Method

- The petroleum ether fraction of *Sarcostigma kleinii* scavenged the DPPH radical in a dose dependent manner with IC_{50} value 480 $\mu\text{g/ml}$. The IC_{50} value of ascorbic acid was found to be 200 $\mu\text{g/ml}$. The scavenging activity of the petroleum ether fraction was lower than that of standard.

b) Nitric oxide scavenging activity

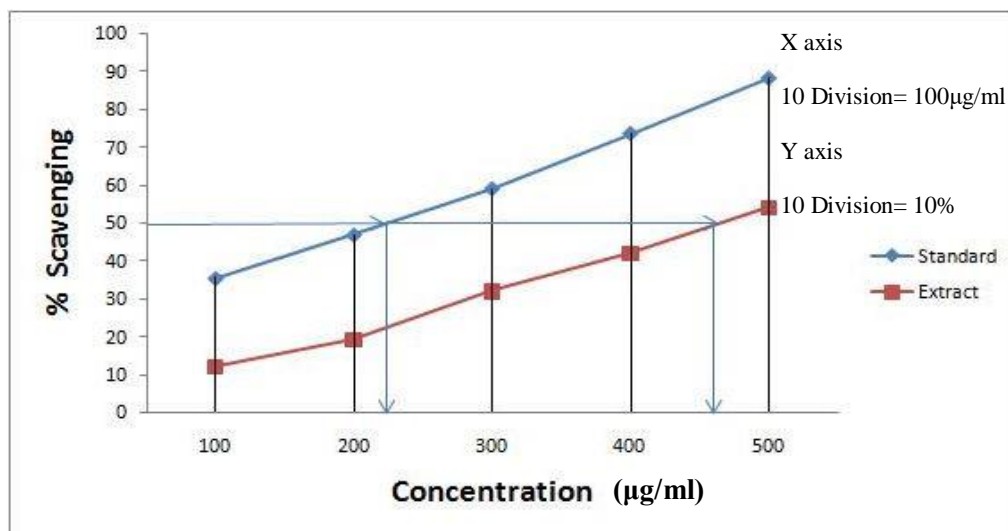
Absorbance of control= 0.984

Table:3 Nitric oxide scavenging activity by standard (Ascorbic acid)

Sl no	Concentration ($\mu\text{g/ml}$)	Absorbance (nm)	% scavenging
1	100	0.625	35.43
2	200	0.514	46.90
3	300	0.396	59.09
4	400	0.255	73.65
5	500	0.114	88.22

Table:4 Nitric oxide scavenging activity by petroleum ether fraction

Sl no	Concentration ($\mu\text{g/ml}$)	Absorbance (nm)	% scavenging
1	100	0.852	11.98
2	200	0.784	19.00
3	300	0.659	31.92
4	400	0.562	41.94
5	500	0.445	54.02



Graph 2: Nitric oxide scavenging activity

- The petroleum ether fraction of *Sarcostigma kleinii* has shown nitric oxide scavenging activity in a dose dependent manner with IC_{50} value 460 $\mu\text{g/ml}$. The IC_{50} value of ascorbic acid was found to be 220 $\mu\text{g/ml}$. The scavenging activity of the petroleum ether fraction was lower than that of standard.

CONCLUSION:

- Preliminary phytochemical screening shows the presence of alkaloids, carbohydrates, phenols, phytosterols, Triterpenoids, flavonoids and tannins.
- Antioxidant study on petroleum ether fraction by DPPH and Nitric oxide method has shown less radical scavenging activity on comparison with standard (ascorbic acid) but has shown significant antioxidant activity.

REFERENCES:

- Sara V, Franca T and Gelsomina F. Traditional uses of medicinal Plants in Valvestino (Italy). J. Ethnoph.2009; 121; page 106-116.
- Ullah R.et al. Traditional uses of medicinal plants in Darra Adam Khel, North western frontier province Pakistan, J.Med Plan Res.2010; 4(17): 1815-1821.
- Sheetal Verma and S.P. Singh Current and future status of herbal medicines Veterinary World. Vol.1 (11); 347-350
- Kamboj, V.P. (2000): Herbal medicine, *Cur. Sc.* 78(1); page 35-39.
- Masood E. Medicinal plants threatened by over-use', Nature. 1997 Feb. 13;385(6617):570.
- Balachandran P, Govindarajan R. Cancer- an ayurvedic perspective, *Pharmacol Res.* 2005; 51;

page 19-30.

- Hamburger M. and Hostettman K. Bioactivity in plants: The link between phytochemistry and medicine, *Phytochemistry.* 1991,page 3864-3874.
- Moronkola Dorcas Olufunke Developments in Phytochemistry, Drug Discovery Research in Pharmacognosy, Prof. Omboon Vallisuta.2012;ISBN: 978-953-51-0213-7. <http://www.intechopen.com/books/drug-discovery-research-in-pharmacognosy/developments-inphytochemistry>.
- Karehed, Jesper . "Multiple origin of the tropical forest tree family Icacinaceae". American Journal of Botany.2001; (12): page no 2259–2274
- Sarr S O et al. *Icacina senegalensis* (Icacinaceae), traditionally used for the treatment of malaria, inhibits in vitro *Plasmodium falciparum* growth without host cell toxicity. *Malar J.* 2011 Apr 11;10(1):8
- Uma G, Balasubramaniam V and Jagathes Kumar S. Evaluation of antimicrobial activity of *Nothapodytes Nimmoniana* Icacinaceae, World journal of pharmacy and pharmaceutical sciences. Volume 2, Issue 6; pages 5078-5083.
- Yesodharan, K., & Sujana, K.A. Wild edible plants traditionally used by the tribes in the Parambikulam Wildlife Sanctuary, Kerala, India. *Natural Product Radiance.*2007; 6(1); pages 74-80.
- Warrier, P K Nambiar, V P K Ramankutty C & Vasudevan Nair R. Indian medicinal plants: A compendium of 500 species. 1996; Volume 5; Pages 76-79.
- Sukhdev Swami Handa et al. Extraction Technologies for Medicinal and Aromatic Plants

- International centre for science and high technology Trieste. 2008; page 102 -105.
15. <https://www.chem.wisc.edu/deptfiles/OrgLab/handouts/CHEM%20344%20TLC%20info.pdf>
 16. <http://web.uvic.ca/~berryde/techniques/column%20chrom.pdf>
 17. Barbara. Stuart Infrared spectroscopy: Fundamentals and applications. Wiley; page 18-24
 18. <http://www.chem.uzh.ch/teaching/documents/master/che431/NMR.pdf>
 19. J Gross. Mass Spectrometry Springer-Verlag Berlin Heidelberg. 2011;Edn 2; page 242-246
 20. Gupta VK, Sharma SK. Plants as natural antioxidants. Nat Prod Rad 2006; 17: 326-334.
 21. Rajesh M P , Natvar J P. *In vitro* antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of Advanced Pharmacy Education & Research .2011;Pages 52-68
 22. Cragg GM, Newman DJ. Plants as source of anticancer agents. J Ethnopharmacol 2005; 100:72-79.
 23. <http://indiabiodiversity.org/species/show/248959>
 24. Karuppusamy Arunachalam Phenolic content and antioxidant potential of *Sarcostigma kleinii* Wight.& Arn, Taylor & Francis.2011; Page no 161-169
 25. <http://eol.org/pages/5228816/overview>
 26. http://www.behr-labor.com/pdf/app_extraktion_gb.pdf
 27. Ajazuddin and Shailendra Saraf Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, a Unani polyherbal formulation Pharmacognosy Res. 2010 Sep-Oct; 2(5): 318–322
 28. Surabhi sharma, Ajay kumar, Ajay G Namdeo Pharmacognostical and phytochemical analysis of *Nothapodytes Nimmoniana* stem, International Journal of Pharmacy and Pharmaceutical Sciences Vol 4, Issue 4, 455-459
 29. Vikrant Arya, Narender Thakur, C.P. Kashyap Preliminary Phytochemical Analysis of the Extracts of Psidium Leaves Journal of Pharmacognosy and Phytochemistry.2012; Vol.1, No.1; page 1-4
 30. Roopalatha U C and Vijay Mala Nair Phytochemical analysis of successive reextracts of the leaves of *Moringa Oleifera* Lam. Vol 5, Suppl 3;page 629-634.
 31. Syed Kamil Mulla and Paramjyothi Swamy Preliminary Pharmacognostical And Phytochemical Evaluation of *Portulaca quadrifida* Linn. International Journal of PharmTech Research. July-Sept 2010; Vol.2, No.3; page no 1699-1702,
 32. <http://bheem.hubpages.com/hub/tlc-thin-layer-chromatography-Principle-Procedure>
 33. [http://courses.chem.psu.edu/chem36/Experiment s/PDF%20s_for_techniques/CC.pdf](http://courses.chem.psu.edu/chem36/Experiment%20s/PDF%20s_for_techniques/CC.pdf)
 34. P. Padmanabhan, S N. Jangle Evaluation of DPPH Radical Scavenging Activity and Reducing Power of Four Selected Medicinal Plants and Their Combinations International Journal of Pharmaceutical Sciences and Drug Research 2012; 4(2); page no 143-14
 35. Gopalakrishnan C K Dhanapal and R Manavalan. *In vitro* antioxidant effect of methanolic extract of root of *coleous vettiveroides*, International Journal of Pharma and Bio Sciences vol 2 issue 4 Oct-Dec 2011 page 353-357
 36. Guno Sindhu Chakraborty. Antioxidant Activity of the Successive Extracts of *Aesculus indica* Leaves, International Journal of Pharmaceutical Sciences and Drug Research. 2009; 1(2); page 121-123
 37. Anurag et al Anti-Cancer Properties of Diethylether Extract of Wood from Sukun (*Artocarpus altilis*) in Human Breast Cancer (T47D) Cells Tropical Journal of Pharmaceutical Research. August 2009;8(4) ;page 317-324.