

International Journal of Innovative Pharmaceutical Sciences and Research

www.ijipSR.com

ANTI-MICROBIAL STUDIES OF *SPERMACOCE HISPIDA* SEED OIL

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ABSTRACT

This paper carried out experimental study, through extraction and characterization of crude *Spermacoce hispida* seed oil. *Spermacoce hispida* belonging to the family Rubiaceae is used in the traditional and siddha system. Seeds used as an astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas, mouth ulcers and abscesses. Petroleum ether, Methanol and water were used as solvent for the extraction process. The characterization analysis revealed that tested parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value for crude oil produced, were within the ASTM standard specifications. Characterization of the oils done based on standard techniques. All the extracts were tested for anti-microbial studies. Anti-microbial studies on gram positive and gram negative organisms reveals that methanolic extract shows significant activity than other solvents which is taken for the extraction.

Key words: *Spermacoce hispida*, seed oil, phytochemical, Anti-microbial.

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INTRODUCTION

There is a great demand for renewable sources of raw materials that have the nutritional and industrial potential. To meet the increasing demand for vegetable oils, improvements are being made with conventional crops as well as with selected plant species that have the ability to produce unique, desirable fats and oils. Seed oils are important sources of nutritional oils, industrial and pharmaceutical importances. *S.hispida* L. (Rubiaceae) was popularly known as “Nattaiccuri” in Tamil or “Shaggy button weed” in English. It is widely distributed in the Western ghats of Kerala and in Maruthamalai forest, which is an extension of Western ghats in Tamil Nadu. The seed-extract of the plant has been used as a remedy for the treatment of internal injuries of nerves and kidney. It is suggested that it can remove signs of old age, purify blood and improve vitality, and has been used by the tribals living in the forest regions in the Western ghats of Kerala since ancient times^[1,2].

Many of the species of *spermacoce* are used traditionally for various diseases like astringent, treating piles, diarrhoea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses. In traditional medicine, *spermacocehispida*s used to heal stomach ailments and also used as tonic, anti-dandruff^[3- 5].

The flowers have been applied to boils, eruptions, swellings, also regarded as an emetic and as a remedy for coughs and malaria. The plant under study has been used since ages by folk because of its rich medicinal values. All the parts of the plant have an ethno medicinal importance. Guided by traditional use of this plant we explored the anti-microbial activity from seed oils.

MATERIALS AND METHODS

Collection and Extraction

The Seeds of *Spermacocehispida* were collected and authenticated from the medicinal garden of Medicinal plants Revitalization and Rehabilitation Centre, Sevaiyur, Tamilnadu. Thirty kilograms of seeds were harvested, air-dried for one week, mixed well and divided into three individual portions of 10kgs each. The seeds were ground to powder using a grinder prior to oil extraction. All chemicals used in the study were analytical grade and used without further purification.

Extraction

Scheme I

The powdered and dried plant material of *Spermacocehispid*(1 kg) was subjected to hot extraction in a Soxhlet continuous extraction apparatus with solvents of increasing polarity viz. Petroleum ether, Methanol. The time period for extraction is 48 h.

Scheme II

Another route of extraction was also followed with 1 Kg of the powdered plant material. It was extracted with petroleum ether, methanol by cold maceration. The individual extracts of both the schemes were filtered and concentrated on Rotary Evaporator.

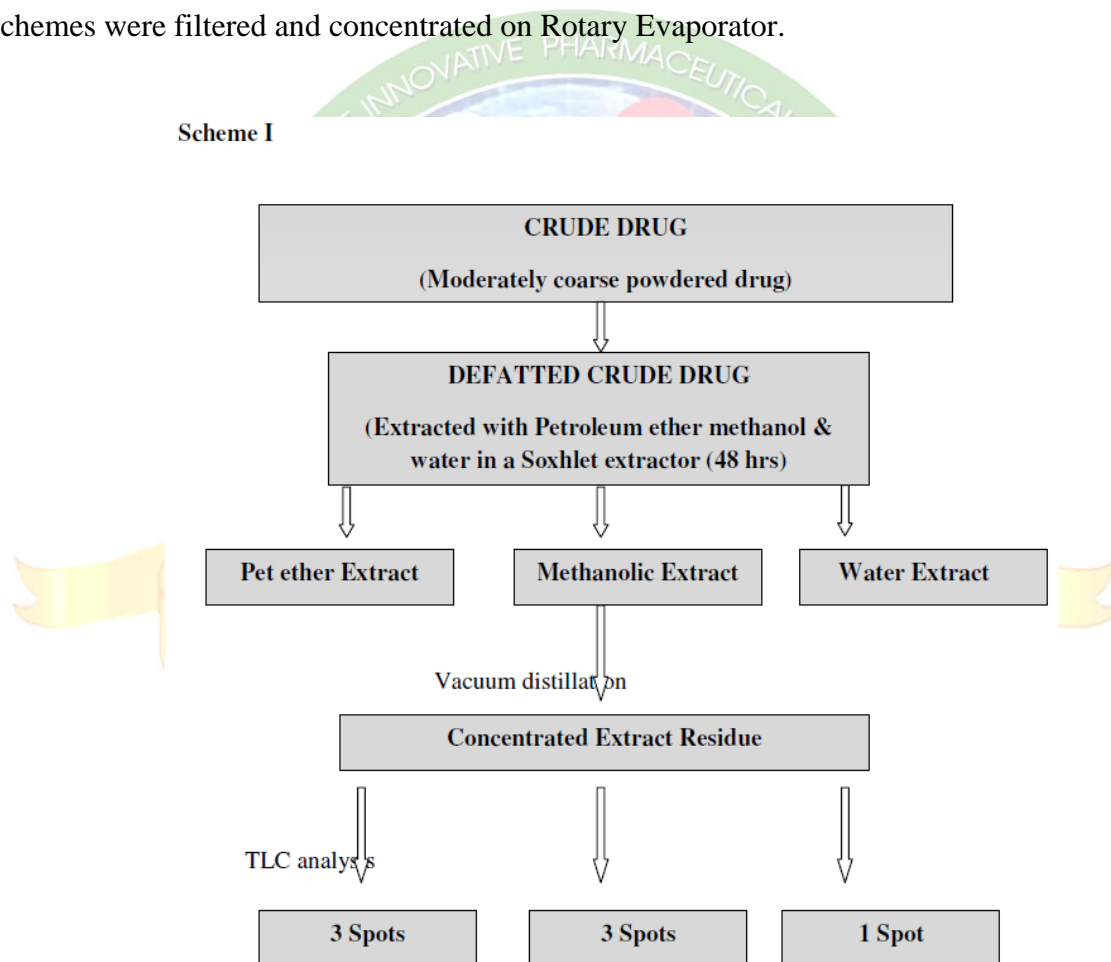


Fig 1: Extraction Scheme

Phytochemical screening

Phytochemical screening of *Spermacocehispidawere* performed by standard procedures viz, Test for alkaloids, Test for reducing sugars (Fehling's test), Test for terpenoids (Salkowski test), Test for flavonoids, Test for saponins, Test for tannins^[6].

Characterization of seed oils

The seed oil characterization analysis performed by following parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value for crude oil produced, were within the ASTM standard specification^[7].

Anti-microbial studies

All the extracts were screened for their antimicrobial activities against *Bacillus subtilis*, *E.coli*, *Candida albicans* and *Aspergillus niger*. Fluconazole was used as the standard drug in the entire test extracts studied in antifungal activity and ciprofloxacin for antibacterial studies. The extracts were studied at concentrations 1000, 500, 250, 125, 62.5 and 31.25 μ g/ml. The zone of inhibition of the extracts was compared with that of standards^[11].

Results & Discussion

Table 1: Preliminary phytochemical investigation

Extract	Alkaloids	Saponins	Tannins	Phenolics	Glycosides	Steroids	Essential oils	Carbohydrates	Flavonoids	Terpenoids
Petroleum ether extract	-	+	+	+	-	+	+	-	+	+
Methanol Extract	-	+	+	+	-	+	+	-	+	+
Aqueous Extract	-	-	+	+	-	+	-	-	+	+

Note: + present. – Absent.

Table 2: Preliminary TLC method development of extracts^[8, 9, 10]

Scheme	Extract	Solvent System	Ratio	R _f values
I	Petroleum ether (60-80 °C)	Benzene : Chloroform	4:1	0.95, 0.68,0.21
I	Methanol	Benzene : Chloroform	4:1	0.85, 0.76, 0.21
I	Water	Benzene : Chloroform	4.1	0.72

Table 3: Results of the analysis of *Spermacocephisida* seed oil

Sr. No.	Parameter	Pet.Ext	Met
1	Seed index	1.540g	1.540g
2	Moisture and volatilities	4.6	4.6
3	Content of seed oil (%)	43.60%	19%
4	Viscosity	9.824	9.9
5	pH Value	7.5	7.8
6	Acid value (mg NaOH g-1 of oil)	1.268	1.46
7	Saponification value (mg KOH g-1 of oil)	194.82	89.60
8	Unsaponifiable matter (%)	1.5	1.1
9	Iodine value (I ₂ g 100 g-1 of oil)	65	45
10	Peroxide value (ml g-1 of oil)	90	36
11	Refractive index (30-40°C)	1.428	1.44
12	Specific gravity	0.962	0.986

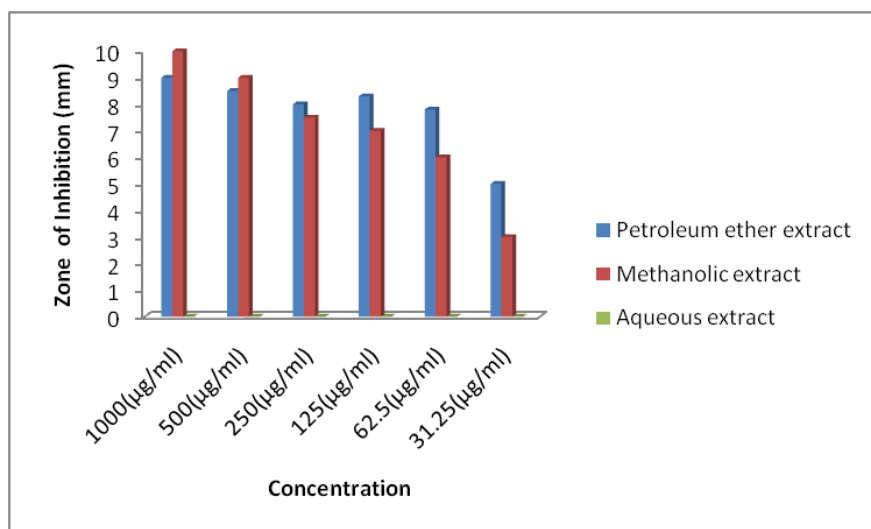


Fig.2: Comparison of Zone of inhibition (Diameter in mm) of petroleum ether extract, methanolic extract and water extract against *Candida albicans* MTCC 227.

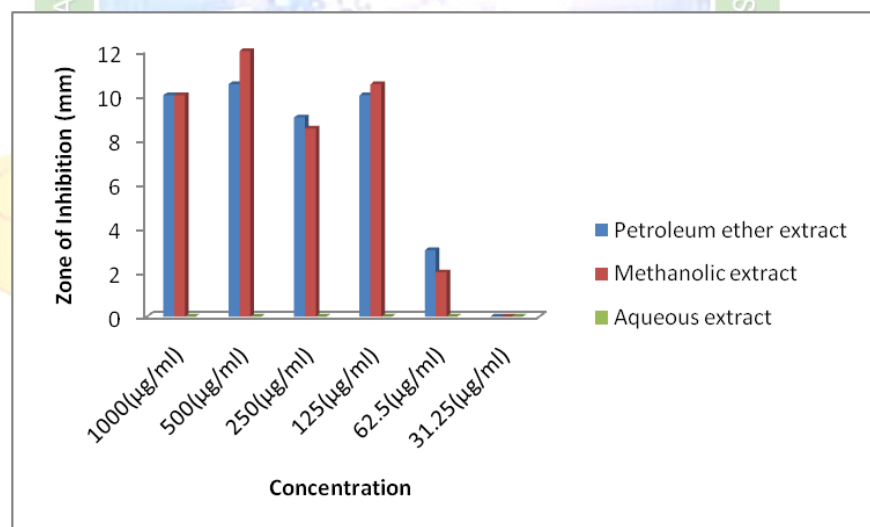


Fig.3: Comparison of Zone of inhibition (Diameter in mm) of petroleum ether extract, methanolic extract and water extract against *Aspergillus niger* MTCC 282.

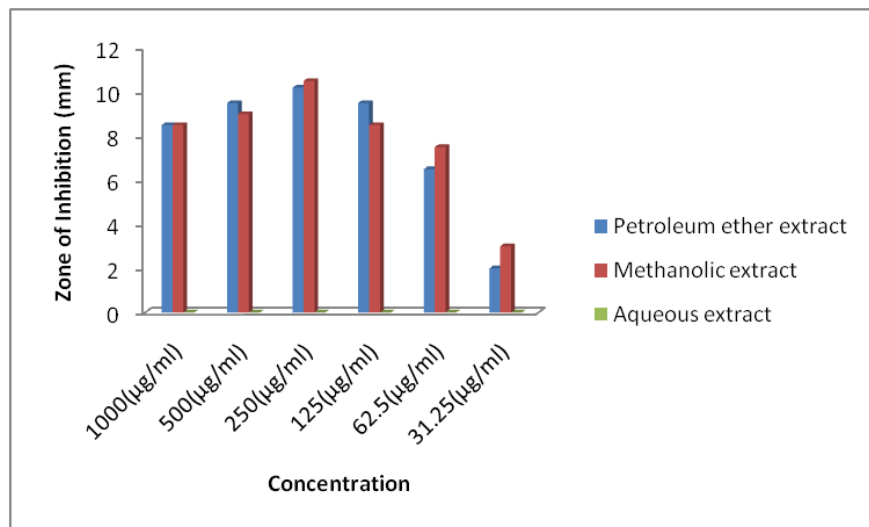


Fig.4: Zone of inhibition (ZOI) of Petroleum ether extract, methanolic extract and water extract against *E.coli* NCIM 2810.

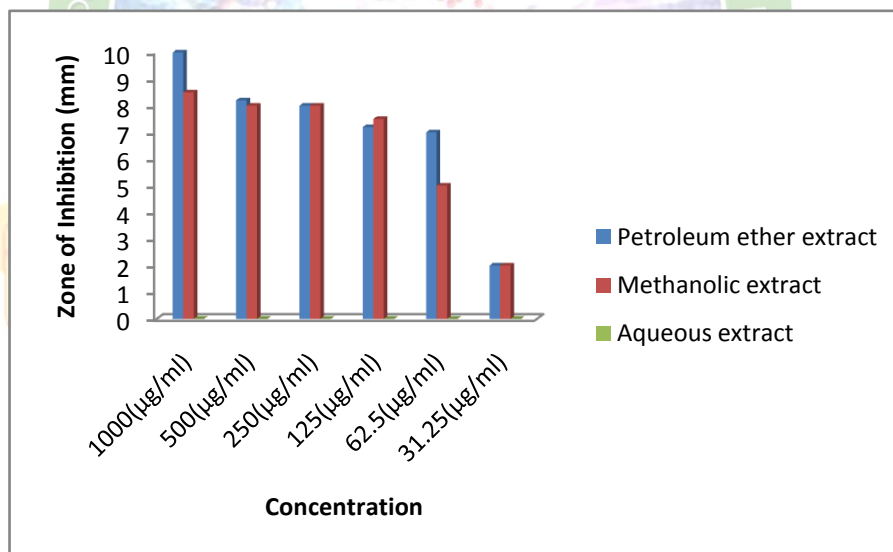


Fig.5: Zone of inhibition (ZOI) of Petroleum ether, Methanolic and aqueous extracts against *B.Subtilis* MTCC441

The results of phytochemical screening indicate that the seeds of *Spermacoccehispid*a contain active phytoconstituents like phenolics, flavonoids, triterpenoids, saponins and steroids. Petroleum ether (60-80°C) and methanol was used as solvent for the extraction process. The seed oil characterization analysis revealed that tested parameters, which include specific gravity, refractive index, acid value, saponification value and iodine value for crude oil produced, were within the ASTM standard specification.

Antimicrobial studies on bacterial and fungal organisms like *Bacillus subtilis*, *E.coli*, *Candida albicans* and *Aspergillus Niger*. The results obtained in this study suggests that the methanol extract of *Spermacoccehispid*a seeds showed more significant activity against on tested fungal and bacterial organism than that of the petroleum ether extract.

CONCLUSION

Having several human diseases and their fatal consequences even after using synthetic drugs, now it is worthwhile to explore natural remedies having lesser side effect and more compatible to the system. This plant viz. *Spermacoccehispid*a has been claimed to treat inflammation, pyrexia, ulcer, bacterial and fungal diseases, malaria and coughs and tumor as per the ethanomedical texts.

The traditional literature suggests that *Spermacoccehispid*a is a plant with high medicinal value, which can be further investigated for other probable pharmacological effects based on traditional uses.

The work on *Spermacoccehispid*a can be extended to other parts also and can be worked upon thoroughly keeping in mind their ethano medicinal importance.

Inspite of the whole-hearted efforts placed at the time of work; it is felt that there is lot of scope for deep Phytochemical studies using modern analytical techniques. The work can be further extended to isolate all other phytoconstituents present in the extracts and also from different type of extracts.

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