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RESEARCH ARTICLE

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PHYTOCHEMICAL SCREENINGAND ANTI-MICROBIAL ACTIVITY OF *HEMIDESMUSWALLICHII*

Nimmagadda Greeshma, Praneetha Srikonda, Saikumar Kunderu, Naredla Kusuma, Khyathi Dondapati and Sindhuja Udumula
Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.

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Abstract

Alternative medicine has become popular these days as itis gaining practices across the globe.The field of alternative therapies is diverse: It encompasses practices spanning diet and exercise changes, hypnosis, chiropractic adjustment, and acupuncture. Ayurvedic medicine is one of the important forms of alternative medicine that was widely available in India. The present study mainly focuseson the identification of therapeutic properties of *Hemidesmus wallichii.* The ethanolic extract of *Hemidesmus wallichii* rootsare used for its anti-oxidant and antimicrobial activity. *Hemidesmus wallichii* dried roots shown good anti-oxidant and anti-microbial properties. The ethanolic extract of *Hemidesmus wallichii* was checked for anti-microbial activity against pathogenic bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas*.

Introduction:-

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in history of all civilizations. Man in the pre-historic era was probably not aware about the health hazards associated with irrational therapy [1]. With the onset of research in medicine, it was concluded that plants contain active principles, which are responsible, for curative action of the herbs. Before onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases [2]. With introduction of scientific procedures, the researchers were able to understand about toxic principles present in the green flora [3,4]. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active [2]. Aconitine, Atisine, Lobeline, Nicotine, Strychnine, Digoxin, Atropine, Morphine are some common examples [5].The efficacy of some herbal products is beyond doubt, the most recent examples being Silybummarianum (silymarin), Artemisia annua (artemesinin) and Taxusbaccata (taxol) [6-8]. On the other hand, randomized, controlled trials have proved the efficacy of some established remedies, for instance, Ginkgo biloba for tinnitus [9]. In Hypericum some researchers are of the view that hypericin is the active principle of the herb and some believe that hyperforin is responsible for antidepressant action of the herb [10]. Recently research has supported biological activities of some medicinal herbs. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease [11].The roots and woody portion havebeen used traditionally for curing various

Corresponding Author:- Nimmagadda Greeshma
Address**:-** Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Guntur, Andhra Pradesh, India.

ailments like stomach pains, fever, veneral disease, rheumatism and act as a blood purifier [12]. And it also possesses antioxidant, antileprotic effects [13]. The present work is based on the standardization of Hemidesmus wallichii root by pharmacognostically as per WHO guidelines. Although other species of this variety has been reported for its standards this has been the complete pharmacognostic validation of this variety which may be used for formulation development in future [14].

Methodology:-

Plant Material

Hemidesmus wallichii root material was selected and made as smoothie for phytochemical screening studies. Root material was dried, powdered and then used for the studies. The procedures recommended in Indian Pharmacopoeia and WHO guidelines were followed to calculate the physico-chemical constants.Physicochemical parameters such as color, consistency, pH and percent yield (% w/w) were determined for all root extracts [15].

Total ash value

The total ash was determined by incinerating 2-3gms of accurately weighed air dried coarsely powdered root in a tarred silica crucible which was previously ignited and cooled before weighing, at a temperature not exceeding 450^0C. The ignition was repeated and the percentage of ash with reference to air-dried drug was calculated.

Water soluble ash

The total ash was boiled for 5min with 25 ml of water. The residue was washed with hot water, ignited for 15min at a temperature not exceeding 450^0C, cooled and weighed. This weight was subtracted from the weight of ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

Acid insoluble ash

The ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5min and filtered through an ashless filter paper. The residue was washed with hot water, ignited, cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

Sulphated ash

The sulphated ash was determined by incinerating 1 gm of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing at a temperature not exceeding 450^0C. The residue was moistened with 1 ml of concentrated sulphuric acid, ignited at $80^0 \pm 25^0$C until all black particles have disappeared. It was then cooled; again, sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air dried drug.The shade dried powdered root material of *Hemidesmus wallichii was* weighed and extracted using 50% ethanol (hydro alcoholic mixture) at 60°C, 50% ethyl acetate at 50°C and methanol at 50°C in soxhlet apparatus and distilled water (aqueous extract) at 100°C for 18 h by hot reflux extraction method. The aqueous, ethanolic, ethyl acetate and methanolic root extracts of *Hemidesmus wallichii* was then filtered and concentrated using rotary vacuum evaporator [16]. The dried root extracts were stored in amber colored wide mouth bottles under refrigeration (4°C) and were used for phytochemical and pharmacological investigations.The preliminary phytochemical investigations were conducted employing variousphytochemical tests and the presence of various phytochemical constituents was detected [17]**.**

Extraction using ethanol and water

Five grams of dried coarse powder of roots were macerated with 100ml of 90% ethanol in a closed flask for 24hrs, shaken frequently for 6 hours and allowed to stand for 18hrs. Filtered immediately taking precautions to prevent loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 105^0C and weighed. The percentage of ethanol soluble extraction was calculated with reference to air dried drug. Five grams of coarse powder was weighed and dissolved in 100ml of water in a stoppered flask, heated at 800C, shaken well and allowed to stand for 10min. It was cooled; 2gms of kieselghur was added and filtered. 5ml of the filtrate was transferred to a tarred evaporating dish and the solvent was evaporated on a water bath. The percentage of water-soluble extraction was calculated with reference to air dried drug.

Determination of volatile oil in drug

Five grams of the drug was boiled with water in a Clavenger's apparatus. The process was continued till no more oil was collected in the graduated tube. The volume of oil was measured and expressed in percentage.

Determination of crude fiber content

About Two grams of the drug was accurately weighed and extracted with ether. Then 200ml of 1.25% sulphuric acid was added and boiled for 30min under reflux. It was filtered and washed with boiling water until free of acid. The entire residue was rinsed back into flask with 200ml of boiling 1.25% sodium hydroxide solution and again boiled under reflux for 30min. The liquid was quickly filtered and the residue was washed with boiling water until neutral, dried at 110^0C to constant weight. It was then ignited to 30min at 60^0C, cooled and weighed. The percentage of crude fiber content was calculated with reference to the air-dried drug.

Determination of loss on drying

Glass stoppered shallow bottle was weighed that had been dried in the same conditions to be employed in the determination. About one gram of the sample was transferred to the bottle and distributed evenly by gently side wise shaking to a depth not exceeding 10mm. Place the loaded bottle in a drying chamber (the stopper was removed and left in the chamber). The sample was dried to a constant weight and allowed to cool. The bottle along with thecontent was weighed. The process was repeated until the successive weights differed not more than 0.5mg (drying to constant weight). The percentage loss of weight was calculated with reference to the air-dried drug.

Determination of foaming Index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml of boiling water. The flask was maintained at moderate boiling at 80-90^0C for about 30min. It was cooled, filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100ml.Ten stoppered test tubes were cleaned (height 16cm, diameter 1- 6cm) and marked from 1 to 10. 1, 2, 3ml up to 10ml of the filtrate was measured and transferred to each tube and adjusted the volume of the liquid with water to 10ml. Then the tubes were stoppered and shaken lengthwise for 15sec uniformly, allowed standing for 15min the length of the foam was measured in each tube.If the height of the foam in each tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the root material is measured and transferred to a 100ml volumetric flask (V2) and the volume is made to 100ml and followed the same procedure.

Fluorescence Analysis

The fluorescence analysis of the drug powder as well as various extracts was carried out by using the method of Chase and Pratt. The behavior of the powder with different chemical reagents was also carried out.

Results and Discussion:-

Ash analysis and moisture contents

In the present study ash analysis for crude powder drug of roots was carried out. It was observed that highest value of total ash was recorded for roots (4.5%). Acid insoluble ash was in the range of 3.25%,Water soluble ash was in the range of 3.34 % (Table 1).Plant species with medicinal properties have been playing a fundamental role in the efforts for drug discovery all over the world. 80% populations in developing countries are dependent on plants for their primary health care, and despite the significant progress in the field of synthetic organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries are derived directly or indirectly from plant sources.

Table 1;- Physico-chemical constants.

S.N	Parameters	Percentage (%w/w)
1.	pH	7.8±0.5
2.	Total ash	5.5±0.3
3.	Acid insoluble ash	4.25±0.4
4.	Water soluble ash	4.44±0.5
5.	Sulphated ash	5.3±0.3
6.	Solubility	
	Water soluble extraction	13.2±0.4
	Alcohol soluble extraction	4.5±0.4
7	Crude fibre content	2.25±0.2
8	Loss on drying	5.56±0.6
9	Foaming index	Less than 100

Table 2:- Phytochemical studies.

S.No	Secondary metabolites	Methanol	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	+	-	+	-
2	Triterpenes	+	+	+	-
3	Saponins	-	-	+	++
4	Tri terpinoidalsaponins	+	-	++	-
5	Alkaloids	+	+	+	+
6	Carbohydrates	-	-	+	-
7	Flavonoids	+	+	++	+
8	Tannins	+	+	+	+
9	Glycosides	-	-	+	-
10	Polyphenols	+	+	++	+

Extraction values

Extractions of crude powder drug with different solvents gives different extraction values. Extraction value is one of the useful methods for evaluation of crude drugs, and provides guidance about the most suitable solvent to be used for extraction, and also helps in detecting various types of adulteration and exhausted materials i.e., Water and alcohol soluble extraction values can be used for the detection of adulterants, defective processing and poor quality of the drug while petroleum ether soluble extraction value indicates lipid contents present in the crude drugs.The fluorescence analysis of powder with various reagents and extracts are given in the Table 3 and 4.

Table 3:- Fluorescence analysis of powder.

S.No	**Reagents**	**Day light**	**Short UV**	**LongUV(365nm)**
1.	Powdered root	Red	Red Dark	Red
2	Powder + 1 N HCl	Yellow	Red	Red
3	Powder + 1 N			
	NaOH	Red	Red	Pale Red
4	Powder + 50%			
	HCl	Yellow	Fluorescent Red	Fluorescent Red
5	Powder + 50%			
	H2SO4	Dark Red	Dark Red	Dark Red
6	Powder +50%			
	HNO3	Dark brown	Brown	Redish brown
7	Powder + ethanol	Red Fluorescent	Red	Light Red
8	Powder + ethanol +			
	1 NNaOH	Red	brown	Red

Table 1:- Antibacterial Activity of extracts

Compound No	Gram negative bacteria		Gram positive bacteria	
	E. coli MTCC 443	*P.aeruginosa MTCC 424*	*S.aureus MTCC 96*	*S.pyogenes MTCC 442*
	Zone of inhibition in mm [b]			
Ethanolic	5	4	5	5
Ethyl Acetate	4	3	3	4
Methanolic	4	3	4	3
Aqueous	1	2	3	1
Ciprofloxacin [a]	5	5	7	6

a. Concentration: 4 mg/mL^{-1} of DMSO; b. Values, including diameter of the well (8 mm), are means of three replicates; c. No activity

Conclusion:-

Phytochemicals present in the different extracts of roots of *Hemidesmus wallichii* was identifiedas prominent source for anti-oxidant property. Among the extracts ethanolic extract has highest anti-oxidant property when compared to other extracts.In the present study it was found that *Hemidesmus wallichii* roots ethanolicextract has an excellent antimicrobial activity. The pathogenic bacteria were inhibited in presence of the root extracts of *Hemidesmus wallichii*. Therefore, the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria.

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