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Phytochemical screening and characterization of *Acorus calamus* for its antimicrobial and antioxidant activity

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Abstract
Ayurveda is one of the traditional medicinal systems of Indian culture. The philosophy behind Ayurveda is preventing unnecessary suffering and living a long healthy life. Ayurveda involves the use of natural elements to eliminate the root cause of a disease by restoring balance and at the same time creating a healthy life-style to prevent the recurrence of imbalance. Herbal medicines have existed world-wide with long recorded history. World Health Organization (WHO) have estimated that 80% of the world's inhabitants still rely on traditional medicines for their health care. India is well-known to be one of the major biodiversity centre with about 45,000 plant species, including 15,000 medicinal plants. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining this research mainly focuses on the importance of polyherbalism and its clinical significance. For this study medicinal plant *Acoruscalamus*have been taken and extracted for their study of anti-bacterial and anti- oxidant activity. The phytochemical compounds were screened by qualitative analysis method and the detected phytochemicals are tannins, saponins, alkaloids, phenols, terpenoids, flavonoids. The different solvent ssuch as methanol, petroleum ether, chloroform and aqueous were used to extract the bioactive compounds from various parts of the selected medicinal plant. The anti- bacterial activity were demonstrated against the bacterial strains like *Staphylococcus aureus* and *Escherichia coli* by disc-diffusion method. The anti-oxidant activity was evaluated by DPPH radical scavenging method.

Keywords: phytochemical screening, anti-microbial activity, anti-oxidant activity, DPPH method, phytotherapy, traditional medicine, polyherbal formulation

Introduction
Nearly 80%of the world's population in developing countries mainly depends on natural products for their health needs (Ramakrishna *et al.,* 1984). Mother earth has bestowed to the mankind and various plants with healing ability for curing the ailments of human being. This unique feature has been identified since pre historic times. The WHO has also estimated that 80% of the world population meets their primary health care needs through traditional medicine only. Medicinal plants are those plants possessing secondary metabolites and are potential sources of curative drugs with the very long list of chemicals and its curative nature. India is the eighth largest country having rich plant diversity with a total of around 47,000 species, of which more than 7500 species are being used as medicinal plants. Plant products are used as main source of medicine throughout the world for treating various human ailments (Balakumbahan R *et al.,* 2010). Chemical composition of alcoholic *A.calamus*extract and essential oil had been investigatedextensively in nearly 20 years. The findings of previous studies demonstrated that the major chemical constituentsof non-aqueous *A. calamus* extract were alpha- and betaasarone. Those bioactive compounds were also found thatresponsible to most of the biological activities includingantioxidant and antibacterial activities. Other than alpha-asarone and beta-asarone, various hydro carbon compounds also identified in *A.calamus*. Phenols andflavonoids are group as type of hydrocarbon due topresence of hydroxyl group attached to the hydrocarbonring on their chemical structure. Infectious diseases are a great burden on many societies. The disease may caused by bacteria, fungi, viruses, protozoa, and multicellular parasites. The infections could spread through skin contact, inhalation, ingestion, transfusion, unprotected sex, and others. The microbes such as *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* are a major human pathogen that causes a wide range of clinical infections. Generally, the pathogens are controlled by Synthetic drugs. However, the drugs are not only expensive and inadequate for the treatment of the diseases but also often have side-effects. The drug side-effects, or adverse drug reactions, have become a major healthcare concern. As an illustration to the extent of this problem, serious drug side-effects are estimated to be the fourth leading cause of death in the US, resulting in 100,000 deaths per year. Friedman *et al.* stated that the spread of resistance in healthcare settings and the community threatens the availability of anti biotic therapy [1-2].

Fig 1: *Acorus calamus*

Acorus calamusis a perennial plant growing to 1 m (3ft 3inch) by 1 m (3ft 3inch). It starts flowering from May to July and the seeds mature from July to August. The flowers are bisexual and are pollinated by insects. The plant prefers clay soils with slightly acidic or alkaline nature. It cannot grow in the shade and requires wet soil; it can also grow in water. The root is emmenagogue, aphrodisiac, stimulant, carminative, diaphoretic, hypertensive, expectorant, and febrifuge, aromatic, hallucinogenic, analgesic, sedative, stomachic and vermifuge. They are used in the treatment of digestive disorders, bronchitis, sinusitis etc. They are said to have excellent tonic powers of stimulating and stabilizing the appetite. Acorus is used externally to treat skin eruptions, neuralgia and rheumatic pains. Chewing the root is said to kill the taste for tobacco [2-3].

Materials and Methods

Sample collection

The whole plant was collected from Govt. Nursery of Ujjain, M.P. India

Preparation of plant extracts

200 ml of solvent (Chloroform, Methanol, Petroleum ether, aqueous) was taken in a round bottom flask. Then 20 gm of drug powder was weighed in a digital weighing machine was wrapped in a filter paper to make a thimble.it was then placed in the central compartment & It was heated at a temperature range between 50^0C-60^0C in a heating mantle. After heating the vapour passes through the side arm up into the reflux condenser. Here the vapour condenses, liquefies & drips into the thimble containing the material to be extracted. The warm solvent percolates through the material & the wall of the thimble & the extract gradually collects in the central compartment. Once the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this & back into the lower round bottomed flask.

Then the process is further repeated as required. In this method the extract gets collected in the lower vessel and gradually becomes more & more concentrated. When the drug powder was completely extracted, the solvent collected in the middle compartment displayed transparent colour. Assuming that there are non volatile substances present, the vapourisation from the heated extract is pure solvent in the vapour form & so the liquid dripped into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed.

The effective volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The extraction process was repeated for Chloroform, Methanol and Petroleum ether [4-5].

Table 1: Phytochemical analysis test chart of acorus calamus (+) --- Positive; (-) --- Negative

Phytochemical Test	Plant			
	Chloroform	Methanol	Petroleum Ether	Aqueous
Alkaliods	-	-	-	+
Flavonoids	-	-	-	-
Tannins	-	+	-	-
Phenols	+	-	+	+
Terpenoids	-	+	-	-
Saponins	+	+	-	+

Anti-bacterial activity by disc diffusion

S. aureus, E. coli and strains were used. 60 ml of Nutrient broth was prepared in 100 ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37^0C for sufficient period of time for organism to grow. After solidification the disc of whatmann filter paper imbibed with 20µl plant extracts were carefully placed with the help of forceps at the centre of the petri dish and then kept in incubator for 24hrs. With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured [3-6].

Antioxidant activity

11 clean test tubes were taken and ascorbic acid solution was added to each of the test tubes in an increasing amount from 0.2,0.4, The eleventh test tube was kept blank with no ascorbic acid. Then methanol was added to make the final volume to 2 ml. Then 0.5 ml of DPPH solution was added to each of the test tubes. The test tubes were allowed to stand for the reaction to occur for 10 min in dark conditions. Finally the readings were noted down by the help of UV VIS SHIMADZU 1800 Spectrophotometer at 517nm. In case of extracts obtained from herbal sample same procedure was used. 20µl of the samples were taken & volume was made to 2 ml with methanol. 0.5 ml of DPPH solution was added to each of the test tubes and it was allowed to stand for reaction for 10 min in dark conditions. Reading was noted down on UV VIS SHIMADZU 1800 Spectrophotometer at 517nm [3]. Determination of percentage inhibition of DPPH Activity by using following formula:

% Inhibition of DPPH Activity = A-B/A *100

Where, A = Optical Density (O.D.) of the blank B = Optical Density (O.D.) of the sample

Results & Discussion

Table 2: Colour of successive extracts of acorus calamus

Name of Reagent	Colour of Extract
Chloroform	Deep red
Petroleum Ether	Cream
Methanol	Purple
Aqueous	Dark green

Table 3: Anti-bacterial activity of chloroform extract of *Acorus calamus*

Extract	Microorganism	Zone of Inhibition (in mm)
Chloroform Extract	*E. coli*	16.5 mm
	S. aureus	19mm
Petroleum Ether Extract	*E. coli*	10mm
	S. aureus	7mm
Methanol Extract	*E. coli*	7.5 mm
	S. aureus	10mm
Aqueous Extract	*E. coli*	No zoi
	S. aureus	5mm
Antibiotics (Penicillin G)	*E. coli*	16 mm
	S. aureus	No Zoi
Antibiotics (Oflaxacin)	*E. coli*	19 mm
	S. aureus	12 mm

The powdered drug was subjected to successive extraction protocol soxhalation. The extract so obtained was tested for

the presence of phytochemical like alkaloid, flavonoids, and Tannins. The anti-bacterial activity of the powder extract was done with Chloroform, methanol and petroleum ether. The results indicate that the anti- microbial activity of the methanolic extract of *acoruscalamus* was comparable with standard antibiotic. This shows the *acoruscalamus* has an anti-bacterial activity and this may be due to the extracted phytochemicals in methanolic extract. But further chemical characterization is needed to confirm the molecule responsible for the activity. The anti-bacterial activity of this herbal formulation was comparable with standard antibiotics like Penicillin G and Oflaxacin.

Anti-oxidant activity of *Acoruscalamus*

Phytochemical screening reveals that the major constituents of *Acorus calamus* extract are phenolic compound, glycosides, alkaloid and flavanoid. Among these phenolic compounds which may be responsible for the activities ofanti-oxidant.

DPPH radical scavenging activity

Acorus calamus had significant scavenging effect on the DPPH free radical which increased with increasing concentration. The scavenging effect of sample was lower than that of Ascorbic acid.

Table 4: Observation table of DPPH method for determining the percentage of inhibition

Sl. no.	Volume of sample (200µl)	Volume of methanol (in ml)	Volume of DPPH (in ml)	Absorbance (at 517 nm)	Percentage (%) of inhibition
01.	Pe Pet. ether	3 ml	0.7	0.172	51.6
02.	Chloroform	3 ml	0.7	0.163	48.6
03.	Methanol	3 ml	0.7	0.208	62.4

Conclusion

The results of this study clearly indicate that *Acorus calamus* have high anti-oxidant activity and radical scavenging activity against various anti-oxidant systems *in vitro*. These assays have important applications for the food and pharmaceutical industry. Moreover, *Acorus calamus* can be used as an easily accessible source of natural antioxidants and as a possible food supplement. In our present study we conclude that *Acorus calamus* has good anti-oxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin and phenolic compounds. It was already reported that naturally occurring phenolic compounds have free radical scavenging property.the presence of alkaloids, falvonoids in extract the extensive survey of literature reveales that *Acoruscalamus* has phytochemical properties the presence of alkaloids and falvonoids, tannis and saponnis in the extract. Flavonoids are most commonly known for their anti-oxidant activity.

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