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Evaluation of Flavonoid, Anthocynin, Tartaic Ester, Antifungal and Antibacterial Activity of Various Polar and Nonpolar Solvent Extract of Ayurvedic Drug

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Abstract: This study is planned to determine the phytochemicals evaluation and antimicrobial activity of different solvent extracts of the prevalent plant *Acorus calamus*. Maximum polyphenolics compound get extracted in dimethyl sulphoxide, acetone, ethanol and methanol extracts where as high amount of anthocynins, flavonol and tartaric ester extraction in dimethyl sulphoxide, ethyl acetate and acetone and alcoholic extracts. Extraction of proanthocyanidins contents is observed in methanol and ethanol solvent extract as compare to other solvent extracts. The *Acorus calamus* was evaluated for its antimicrobial activity using a disk diffusion assay of methanol, ethanol, actone, ethyl acetate, actonitrril, trichloromethane, dichloromethane, water, tolune, bezene, hexane, dimethyl sulphoxide extracts resulting in the inhibition of a number of bacteria. It was found that all extracts showed pronounced inhibition on gram positive and Gram negative bacteria and fungus strains like *B.subtilis, Ecoli* and *Aspergillus niger.* The antimicrobial activities increased with increasing respective extracts concentration, but did not differentiate significantly in terms of the effect of extraction solvents. When various, pronounced antimicrobial activity on gram positive bacteria methanol, ethanol, actone, ethyl acetate, actonitrril, tri chloromethane, dichloromethane, water, tolune, bezene, hexane, dimethyl sulphoxide extracts suggest that maximum antibacterial activity was found for ethanol, methanol, ethyl acetate and dimethyl sulphoxide extracts. Where as high antifungal inhibition observed for ethanol, acetone, acetonitril extracts.

Keywords: Phytochemicals, Polyphenolics, Antifungal activity, Antibacterial activity, *Acorus calamus.*

INTRODUCTION

In asian country before BC people are aware regarding infections & noninfectious disease & drugs variety of treatment had been given by practitioner which is mainly based on plants & acupuncture therapy which can be suggested by Ayurveda, unani etc. religious books on the basis of that various country are giving educations to people to over about traditional medicines (Bebb, 2011).

As a consideration of this many researcher are working for findings of pharmacological discovery of the various medicinal plants (Kennedy and Wightman, 2011). In support of traditional medicine various invitro & invivo. The studies had carried out. Result obtained from these studies had been discovered many drugs from plants. Various disease like antimicrobial, cancer, neurological disorders, cardiovascular viral etc. Still there are too much work is required to various medicinal applications of the medicinal plants.

Acorus calamus is herb categories plants has various traditional medicinal application which was described by various reports & books. *Acorus calamus* is used for gout rheumatism, gastro intestinal track, small pox, mental disorders, cough etc. diseases. Presence of broad spectrum application researcher had studied for, central

nervous system, behavioral changes, antimicrobial, anticancer, antinuclear, antioxidant, polymerass & acetylcholine inhibitor activities (Kennedy and Wightman, 2011; Mehrotra et al., 2003; Pawar et al., 2011; Mehrotra et al., 2003; Vohora, Shah and Dandiya, 1990; Phongpaichit et al., 2005). In various extracts of *Acorus calamus* put forth of several activities of infectious and non infectious diseases. Current research reports focused on analysis of pharmaceutical active compounds, antibacterial and antifungal studies of *Acorus calamus* polar & nonpolar solvent extracts.

Material and Method

Methanol, Ethanol, Actone, Ethyl Acetate, Actonitrril, Tri chloromethane, Dichloromethane, water, Tolune, Bezene, Hexane, Dimethyl sulphoxide, HCL, agar, nutrient broth, *Acorus calamus* powder etc.

Extraction of phytochemicals

1gm of *Acorus calamus* powder was mixed with 15 ml of solvents such as Methanol, Ethanol, Actone, Ethyl Acetate, Actonitril, Tri chloromethane, Dichloromethane, Water, Tolune, Bezene, Hexane, Dimethyl sulphoxide and kept it for steering at 37^0C for 24 hours.

Analysis of Phenolics

Analysis of total polyphenolics, tartaric ester, flavonol and anthocynins of extracts was estimated with modification in previous method (Fukumoto and Mazza, 2000; Kaur and Kapoor, 2002). The addition of reagent consisted of insertion of 0.10 mL of sample or standard in a test tube followed by 0.25 mL of 0.1% HCl in 95% ethanol and 4.55 mL of 2% HCl. The solution was mixed and allowed to sit for approximately 15 min before reading the absorbance at 280, 320, 360, and 520 nm with a spectrophotometer. The absorbance at 280 nm was used to estimate total phenolic content, 320 nm was used to estimate tartaric esters, 360 nm was used to estimate flavonols, and 520 nm was used to estimate anthocyanins. Standards used were gallic acid in 10% ethanol for total phenolics, caffeic acid in 10% ethanol for tartaric esters, quercetin in 95% ethanol for flavonols, and malvidin-3-glucoside in 10% ethanol for anthocyanins.

Antimicrobial activity

The determination of the inhibitory effect of the *Acorus calamus* extracts on test bacteria and fungus was carried out by disc diffusion method. microbial cultures were grown at 37^0C for 24 h in Nutrient Broth. The culture suspensions were adjusted by comparing against 3.0 McFarland. Petri dishes with 10 ml of Nutrient Agar were prepared, previously inoculated with 100 uL of the culture suspension. The discs (6.0 mm) were made and the extract which is dissolved in DMSO was added on disc and same volume of DMSO was used as a control. The plates were incubated at 37 °C for 24 h for culture of microorganisms. After incubation, the growth inhibition zones around the discs were observed, which indicated that the examined extracts inhibited the growth of microorganisms. After incubation, the diameter of the inhibition zone was measured with calipers. Each assay in this experiment was repeated three times (Maksimović, Malenčić and Kovačević, 2005).

Result and Disscussion

Phytochemicals analysis

Table 1: UV-Spectrophotometric study of different solvent system phenolic content, tartaric esters, tartaric esters, flavonols and anthocyanins

EXTRACT	280nm total Phenolics	320nm Tartaric esters	360nm Flavonols	520nm Anthocyanins
ETOH EX	1.1785	0.3903	0.283	0.0183
MEOH	0.877	0.3384	0.2521	0.0189

ETHYL ACE	0.8138	0.2299	0.1759	0.0276
ACTONE	2.4393	0.3738	0.0184	0.0236
HEXANE	0.0913	0.0389	0.0253	0.0123
H20	0.174	0.0601	0.0341	0.0117
DCM	0.1818	0.0663	0.0665	0.0121
TCM	0.4363	0.0624	0.0612	0.0078
BEZ	0.3223	0.0856	0.0837	0.0076
ACNT	0.7306	0.1921	0.1573	0.0118
TOLUNE	0.5863	0.1096	0.0999	0.0106
DMSO	1.384	0.6121	0.4055	0.0665

Spectrophotometric analysis were conceded out to get approaching into the polyphenolic and its derivatives profile of *Acorus calamus* extracts of different solvents extraction. The results for total polyphenolics and its derivatives of phenolics are presented in Table 1. The research showed differences in the mass amounts of the total phenolic compounds, flavonoids, flavanols and anthocyanins (Cordell, 2002). The obtained results are confirmation of the extraction solvent dependence on the content of not just total polyphenols but individual phenolic derivaties also. The contents of identified individual suggest that DMSO, alcoholic, acetone, ethyl acetate and acetonitril solvent extractions has small difference in extracting the phytochemicals compared to content of other solvent extraction efficiency. However, as these polyphenols, especially flavonoids and flavonols are the compounds postulated for health benefits of natural products. All estimated phytochemicals have potential regarding content of these biologically activities such as antioxidant, antimicrobial anticancer etc. (Kennedy and Wightman, 2011; Wang et al., 2010, Du et al., 2003; Fang and Casida, 1999)

Table 2. Antimicrobial activity of extracts against ***B. subtilis***

Sr.no.	Sample code	5 conc. In µl	10 conc. In µl	15 conc. In µl	20 conc. In µl
1	MeOH	8	9.5	11	12
2	EtOH	8.5	11	12.5	14.5
3	ACTONE	8	9.5	11	12
4	ETHYL ACETATE	7	10	11	12.5
5	ACTONITRRIL	7.5	9.5	11	12
6	TCM	NI	6.5	9	12
7	DCM	NI	NI	8	9
8	H2O	NI	7	8.5	9.5
9	TOLUNE	6.5	9	10	11
10	BEZ	NI	7.5	9	10.5
11	HEX	NI	6.5	7.5	10
12	DMSO	7	10	11	12

Table 3 . Antimicrobial activity of extracts against ***Ecoli***

Sr.no.	Sample code	Control 5µl	5 conc. In µl	10 conc. In µl	15 conc. In µl	20 conc. In µl
1	MeOH	NI	7	8.5	10	13
2	EtOH	6.5	7	9	11	13
3	ACTONE	NI	6.5	8	10	11
4	ETHYL ACETATE	7	6.5	7	8.5	10
5	ACTONITRRIL	7	7.5	8.5	10	12
6	TCM	NI	7.5	9	10	11.5
7	DCM	6.5	6.5	8	9.5	10
8	H2O	NI	6.5	8	9.5	11

9	TOLUNE	NI	8	10,10	9.5	12
10	BEZ	6.5	8		9	11
11	HEX	NI	6.5	7.5	9	10.5
12	DMSO	6	6.5	7.5	8	10

Table 4. Antimicrobial activity of extracts against ***Aspergillus niger***

Sr.no.	Sample code	Control 5µl	5 conc. In µl	10 conc. In µl	15 conc. In µl	20 conc. In µl
1	MeOH	6.5	8	9	12	13
2	EtOH	7.5	10	15	15	15
3	ACTONE	6.5	9	8	10	14
4	ETHYL ACETATE					
5	ACTONITRRIL	7	9	11	10	14
6	TCM	NI	7	8	9	11
7	DCM	NI		9	10	12
8	H2O	NI	NI	NI	NI	NI
9	TOLUNE	NI	NI	NI	NI	NI
10	BEZ	NI	NI	NI	NI	NI
11	HEX	NI	8	10	10	13
12	DMSO	NI	8	9.5	11	11.5

Antimicrobial activity

Infectious diseases represent a serious health problem generally worldwide and today also. The problem arises mostly due to the appearance of antibiotic resistant strains. This situation is more complicated in the world as a outcome of the relative poverty of the population, explaining why more people are dependent on plant-based drugs *Acorus calamus* is one of the most broadly used spices in traditional medicine (Vohora Shah and Dandiya, 1990). Its antimicrobial activity against both Gram - positive and Gram-negative bacterial species was demonstrated. In the present study, all the extracts compounds were tested against microbial strains such as *Escherichia coli, B.subtilis* and *Aspergillus niger* using disk diffusion methods. For the purpose of easier visualization, the zone date from these assays indicates the average diameter (from 3 trails) of the growth inhibition zones. The margin of error of these measurements is ±1 mm. The antibacterial activity was classified as highly active (>14 mm), moderately active (10–14 mm), slightly active (6–10 mm) and less than 6 mm was regarded as inactive. The results of our antibacterial and antifungal studies of all the extract were depicted in Table 2, Table 3 and Table 4. From Table 2, Table 3 and Table 4, it can be observed that Maximum antibacterial activity was found for bacillus subtilus inhibition were observed for ethanol, methanol, ethyl acetate and DMSO extracts. Remarkable growth inhibition of *Ecoli* observed for methanol, ethanol and acetonitril extracts. Ethanol, acetone, acetonitril extracts exhibit the good antifungal activity against *Aspegillus niger.*

Conclusion

In the present study, a phytochemicals was extracted from *Acorus calamus* by using various solvents while its antimicrobial activities were evaluated. The study indicated that the polar solvents extracts exhibited high content of Phytochemicals and antimicrobial activities as compare to non polar solvent extracts. Further work about the isolation's of respective Phytochemicals and their activities using in vivo models is worth investigation for future drug discovery and drug formulations.

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