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Research Article

PHYTO CHEMICAL, ANTI FUNGAL, ANTI MICROBIAL AND ANTI OXIDANT STUDIES ON WHOLE PLANT EXTRACT OF PENTATROPIS CAPENSIS

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ABSTRACT

The genus pentatropis is considered as one of the most important genus used in various systems of medicine due to their reported variety of compounds. In the present investigation the plant, *Pentatropis capensis*, was examined chemically for its phytoconstituents. Investigation revealed the positive results for the presence of steroids, flavanoids, tannins and glycosides. The chloroform extract of stem showed moderate activity against bacterial organisms like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli.* The chloroform extract of the whole plant showed no antifungal activity at concentrations of 100mg/ml, 300mg/ml against fungal organisms. Methanollic extract of the plant showed significant effect on hydrogen peroxide radical scavenging activity and less significant effect on nitric oxide radical scavenging activity when compared with the standard.

Keywords: Pentatropis capensis, Scavenging activity, Gram + Ve, Gram –Ve.

INTRODUCTION

In the last few decades there has been and exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. In olden times, *vaidyas* used to treat patients on individual basis, and prepare drug according to the requirement of the patient.Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology, and phytotherapy. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts[1].

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated - a number

estimated to be less than 10% of the total. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds[2,3].

Phytochemical examination of the whole plant of *Pentatropis capensis*

A survey of literature revealed that no phytochemical study has been reported on whole plant of Pentatropis capensis, keeping in view of the medicinal importance of the genus and varied compounds reported, the author felt it worthwhile to examine chemically, the whole plant of the above species for their phytoconstituents.

The plant ia a slender climber with leaves of sizes 2-3.5 x 1-2.2 cm, thick, ovate, obtusemucronate at apex, subcordate at base, glabrous or nearly so; flowers are umbellate cymes; follicles 4-6 x 0.6-0.8 cm, lanceolate, beaked, glabrous.[17]The plant was shown in fig.1

EXPERIMENTAL

The air dried whole plant (411g) of Pentatropis capensis were powdered in a wiley mill and extracted with chloroform (3 lits each) for 6 hrs respectively. The procedure was repeated for 5 times. The extracts were concentrated under vacuum to get the corresponding residues (7.4gm). It gave the following chemical tests and was presented in table[1].The chloroform extract residue on TLC showed 4 spots in 1:1 chloroform:methanol solvent system. The chloroform extract was column chromatographed over silica gel (100-200 mesh) and eluted with petroleum ether, benzene, chloroform and methanol in order of polarity and 250ml fractions were collected. Each fraction after concentration was monitered on TLC.

1. Antibacterial activity of the *Pentatropis capensis* whole plant extract

The antimicrobial activity of any sample of natural origin is assayed separately using the agar diffusion method employing 24 hours cultures of test bacteria and fungi strains. To carry out these studies, disc diffusion method and agar cup plate method are to be routinely adopted. However, in the present studies only the later method has been chosen. The effect of the extract and the standard drug were analyzed by measuring the diameter of inhibition zones in millimeter are recorded.

Method used for the experiment: Cup-Plate Agar Diffusion Method

Extract and dosage used in the experiment: chloroform extract of the whole plant of Pentatropis capensis was used in two dose levels of 100mg/ml and 300mg/ml.

Test organisms

Gram (+) ve bacteria: *Staphylococcus aureus*, *Bacillus subtilis*,.

Gram (-) ve bacteria: *Escherichia coli*

Composition of Nutrient Agar Medium

Component	Quantity
Peptone	5g
Sodium chloride	5g
Meat extract	5g
Agar-agar	20g
Distilled water	Upto 1000ml
PH	7.22-7.4

Procedure

28g of powdered nutrient agar medium (Hi-Media) was suspended in 1000ml of hot distilled water. The prepared nutrient agar medium was sterilized in autoclave at 120°c (15 lb/sq.in) for 20 minutes. 25 ml of the nutrient agar medium prepared was inoculated with 24 hours old stock cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter Petri dishes. The medium in the plates were allowed to set at room temperature for about 30 minutes to solidify in laminar air flow unit. 4 cups of 6mm diameter were made in each plate at equal distance. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml and 300mg/ml. 50µl of each concentration were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard Antibiotic chloramphenicol (1mg/ml) was used as standard. The Petri dishes thus prepared were incubated for 24hrs at 30°C and were later examined by measuring the zones of inhibition in millimeters and the results were tabulated in the table 4**.**

RESULTS

From the above observations, the chloroform extract of the stems *of* Pentatropis capensis showed moderate activity against Gram (+)ve and Gram (-)ve organisms *staphylococcus aureus, streptococcus anginosus , Bacillus subtilis, Micrococcus luteus, Lactobacillus acidophilus, streptococcus mutans, Proteus vulgaris, Erwinia, Enterobacter aerogens, E.coli, Pseudomonas aeuriginosa , Klebsiella pneumonia* the increase in the extract concentration will results in increase in the anti bacterial activity.

2. Anti-fungal activity of the whole plant extracts of *Pentatropis capensis:*

Test samples: The antifungal activity of chloroform extract of the whole plant pentatropis capensis was performed using Agar cup plate method. The chloroform extracts of the whole plant pentatropis capensis in two dose levels of 100mg/ml and 300mg/ml.

Test organisms: Aspergillus niger**,**Rhizopus stolonifer**,**Mucor recemosus

Composition of PDA medium[18]

Component	Quantity
Potatoes(peeled)	200g
Dextrose	20g
Agar-Agar	20g
Distilled water	Upto 1000ml

Procedure: 200g of Peeled potatoes were cut into small pieces and boiled with 500ml of water for 30 minutes. The pieces were crushed during boiling and the pulp was removed after cooling by filtration through muslin cloth. To the filtrate, required quantities of dextrose was added and the volume was made up to 1000ml. Boil it on water bath, then add agar-agar on stirring continuously for 5minutes, and distributed in 20ml quantities into boiling tubes and was sterilized in autoclave at 121º(15lbs / sq.in) for 20 minutes. The medium was inoculated using 2days culture of the organisms in aseptic conditions and transferred to sterile Petri dishes

of 15cm diameter. The medium in the plates was allowed to set at room temperature for about 30min.25 ml of the nutrient PDA medium (Hi-media) was prepared and inoculated with 5 micro liters of aqueous suspension of the above mentioned test organisms, which were prepared from 48 hours cultures, are thus transferred into sterile Petri dishes. The medium in the plates were allowed to set at room temperature for about 10 minutes. 4 cups of 5mm diameter were made in each plate at equal distances. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml and 300mg/ml. 50µl of each of the above stock concentrations were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard. Fluconozole (10mg/ml) is used as reference standard. The Petri dishes thus prepared were incubated for 48 hours at 37° C and were later examined by measuring the zones of inhibition in millimeters.

RESULTS

The antifungal activity of chloroform extract of the whole plant pentatropis capensis has no activity at 100mg/ml, 300mg/ml concentrations on the tested organisms.

3.Anti oxidant activity of whole plant extract of *Pentatropis capensis*

Introduction

Antioxidants are substances that may protect your cells against the effects of free radicals. Free radicals are molecules produced when your body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases.[19]

Method of assay[21]

(a) Hydrogen peroxide scavenging assay

Chemicals: Hydrogen peroxide,phosphate buffer (ph 7.4), Gallic acid and extract.

Apparatus: Spectrophotometer and p^H meter.

Preparation of standard solution: Required quantity of Gallic acid was dissolved in to give (10, 20, 30, 40, 50) µg/ml

Preparation of Hydrogen peroxide solution: Required quantity of Hydrogen peroxide is dissolved in phosphate buffer to give 100mM solution with Ph 7.4

Preparation of sample solution: required quantity of sample was dissolved in
Phosphate buffer to give (100, 200, 400, 600, 800, 1000) µg/ml

Procedure

2ml of Hydrogen peroxide solution is taken and to it, 1ml of the standard Gallic acid of various concentrations are added.
2ml of Hydrogen peroxide solution is taken and to it, 1ml of the extract of various concentrations are added.
The above-prepared solutions are incubated for 10min.
Absorbance was taken at 230nm using phosphate buffer as a blank.

Hydrogen peroxide scavenging activity

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus removal of hydrogen peroxide radical is very important for protection of food system. in the experiment the scavenging ability of

extraction Hydrogen peroxide free radical was shown in Table[5] and are plotted against standard by a bar chart .As seen from figure the activities of all the samples increased in concentration dependent manner. The comparison standard Gallic acid showed valuable high radicals scavenging activity (97.85%) in the higher doses.

RESULTS

The methanol extract of Pentatropis capensis has shown significant effect on hydrogen peroxide radical scavenging activity.

(b) Nitric oxide scavenging activity

Chemicals required

Sodium nitroprusside, phosphate buffersaline, sulfanlic acid, napthaline diamne dihydrochloride.

Preparation of reagents

Sulfanilic acid reagent: 20% of glacial acetic acid was prepared by dissolving 20ml of glacial acetc acid in 100ml of distilled water , 0.33ml of sulfanilic acid is taken and is make up to 100ml wth 20% glacial acetic acid.

Napthyl Ethylene Diamine Diamine Dihydro Chloride(0.1%w/v): 0.1gm of napthyl ethylene damine dihydrochloride is dissolved n 100ml of distlledwater.

Sodium nitroprusside(10mm): Molecular weight is 297.95gm n 1000ml = 1ml.

29.795gm in 1000ml = 0.1 ml, 2.9795gm in 1000ml= 0.01ml, 0.29795gm in 1000ml= 0.001ml, 0.2979 gm in 100ml =10mm.

Preparatio of Aqueous Extract

100gms of crude powder is taken n stainless vessel and mixed wth 2000ml (1:20) of distlled water , it is then boiled for 2hrs using gas burner and filterate is poured in borosilicate 500ml beaker and is then evapourated on hot plate untill it reaches the concentrated quantity (do not be in viscous state).

Procedure

Ntric oxide radical inhibiton was estimated by usng napthyl ethylene d amine d hydrochloride (0.1%w/v).The reaction mixture containing 2ml of 10mm sodium nitroprusside ,0.5ml saline phosphate buffer and 0.5ml of standard solution (taken as ascorbic acid or quercetin)and aqueous ethanolic extract of 500-1000 µg/ml are incubatedat 25 ˚c for 150 mins.After incubaton ,0-5ml of the above reaction mixture was mxed with of sulfanlc acd reagent (0-33% n 20% glacial acetic acid) and allowed to stand for 5mins for the completion of the reaction of diazotzation.After that 1ml of napthyl ethylene diamine hydrochloride was added, mixed and was allowed to stand for 30mins at 25˚ c .The concentration of nitrile was assayed at 540nm and was caliculated wth the control absorbance of the standard nitrle solution .

Here, the blank is taken as buffer and make up solvents and ascorbic acid and quercetin was taken as standard.

% Scavenging activity={(Acontrol- Atest or Astd)}*100

Where , A control=Obsorbance of control

A test or A std = Absorbance of test or std.

Nitric oxide scavenging activity: Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes increasing lipid peroxidation and development of insulin resistance. These consequences of oxidative stress can promote the development of complication.

Nitric oxide (NO) unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates through NO_2, N_2O_4 and N_3O_4.is a potent pleiotropic inhibitor of physiological processes such as smooth musclerelaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities. Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates through NO_2, N_2O_4 and N_3O_4. It is estimated by using the Griess reagent. In the presence of test compound, which is a scavenger the amount of nitrous acid will decrease. Compared to the standard of Ascorbic acid showed valuable high radicals scavenging activity (93.85%) in the higher doses. Results are shown in table [6] along with its comparison plot with the standard.

RESULTS

The methanolic extract of Pentatropis capensis has shown less significant effect on nitric oxide radical scavenging activity when compared with standard.

Fig. 1

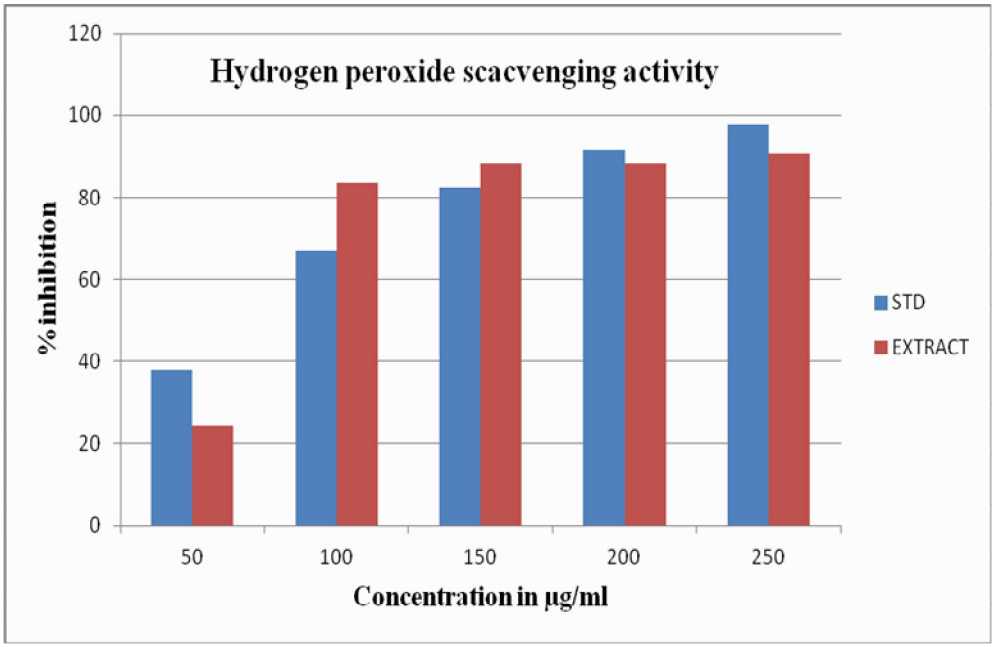

Fig. 2: Pentatropis capensis

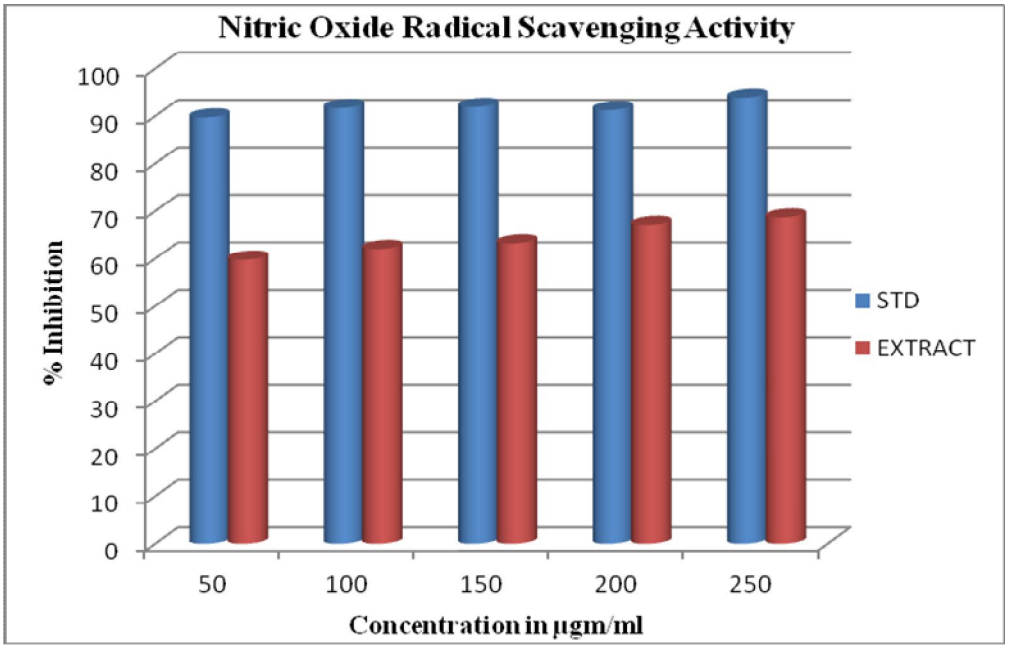

Fig. 3

Table 1:Chemical tests for the chloroform extract of whole plant of *Pentatropis capensis*

Name of the test	*Pentatropis capensis* chloroform extract
Steroids:	
A)Salkowski Test:	(+) ve
B)Liebermann Buchard's Test:	(+) ve
Triterpenes:	
A) Salkowski Test:	(-) ve
B)Liebermann- Buchard's Test:	(-) ve
Alkaloids:	
A)Mayer's Test:	(-) ve
B)Dragendroff's Test:	(-) ve
C)Wagner's Test:	(-) ve
D)Hager's Test:	(-) ve
Carbohydrates	
A)Fehling's Test:	(+) ve
B)Molisch's Test:	(+) ve
D)Barfoed's Test:	(+) ve
C)Benedict's Test:	+) ve
Flavonoids:	
A)Shinoda Test:	(+) ve
B)Ferric chloride Test:	(+) ve
C)Lead Acetate Test:	(+) ve
D)Zinc- Hcl reduction Test:	(+) ve
Tannins:	
A)Ferric Chloride Test:	(+) ve
B)Gelatin Test:	(+) ve
Glycosides:	
A)Baljet Test:	(+) ve
B)Legal Test:	(+) ve
C)Keller- Killiani Test	(+) ve
D)Kedde's test:	(-)ve

Table 2:Antibacterial activity of chloroform extracts of the whole plant of Pentatropis capensis

S. No.	Name of the extract	Diameter of zone of inhibition		
		Gram +Ve bacteria		Gram +Ve bacteria
		S.A	B.S	E.C
1	Chloroform extracts of *Pentotropis capensis*(100mg/ml)	10	18	10
2	Chloroform extracts of *Pentotropis capensis*(300mg/ml)	11	19	12
3	Standard	27	50	20
4	Control	-	-	-

Zone of inhibition in millimeters, cup diameter: 6mm
S.A: *Staphylococcus aureus*;
B.S : *Bacillus subtilis;* E.C : *Escherichia coli*

Table 3:Hydrogen peroxide scavenging activity

S. No.	Concentration(µg/ml)	Absorbance	% Scavenging activity
	Control	1.211	-
		Gallic acid	
1.	50	0.752	37.90
2	100	0.397	67.21
3	150	0.211	82.57
4	200	0.101	91.65
5	250	0.026	97.85
		Pentatropis capensis	
1	50	0.917	24.27
2	100	0.199	83.57
3	150	0.143	88.19
4	200	0.142	88.27
5	250	0.110	90.91

Table 4:Nitric oxide scavenging activity

S.No.	Concentration (µg/ml)	Absorbance	% Scavenging activity
	Control	1.655	-
		Ascorbic acid	
1	50	0.170	89.72
2	100	0.137	91.72
3	150	0.133	91.96
4	200	0.114	91.29
5	250	0.100	93.95
		Pentatropis capensis	
1	50	0.699	59.77
2	100	0.628	62.05
3	150	0.608	63.26
4	200	0.545	67.06
5	250	0.519	68.64

CONCLUSION

All the above studies revealed the extract of the whole plant of pentatropis capensis has significant antibacterial activity against both gram +ve & gram –ve bacteria.It is aslo having hydrogen peroxide scavenging activity & nitric oxide scanvenging activity.

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