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**Phytochemical studies and biological activities on fruits of
*Momordica Cochinchinensis***

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ABSTRACT

The methanolic extract of *Momordica cochinchinensis* was subjected to preliminary phytochemical studies. The results indicate the presence of glycosides, flavonoids, carbohydrates and phytosterols. Antimicrobial activity was screened by using agar well diffusion method. The results revealed that the methanolic extract exhibited significant anti-microbial activity at concentration of 100-500 µg/ml respectively against tested organisms, particularly more effective against gram(+)ve bacteria *Staphalococcus aureus*, and gram (-) ve bacteria *Esherichia coli*. than the aqueous extract when compared to the standard drug (Ampicillin). Antioxidant activity by DPPH method shows better results for test drug when compared to the standard drug (ascorbic acid).

Key Words: *Momordica cochinchinensis*, anti-microbial activity, DPPH method, phytochemical studies.

INTRODUCTION

Momordica cochinchinensis is a relatively short harvest season (which peaks in December & January) it is typically served at ceremonial or festive occasions in Vietnam which are commonly grown on lattices cultivated in gardens through out India[1-5]. Gac has shown to be especially high in lycopene content and also contains a protein that may inhibit the proliferation of cancer cells and also beta-carotene which may bound to long chain fatty acids resulting in more bioavailable form with several phytonutrients, vit-E, fatty acids, carbohydrates, flavonoidal glycosides. This fruit is used as both food and medicine and also promote healthy vision by relief of dry eyes. It also contains antioxidant, anti-microbial and antidiabetic .properties.[6-9]

EXPERIMENTAL SECTION

Plant material

Normally the Gac grows on dioecious vines and is usually collected from fence climbers. The fruits were collected in October 2010 from the surroundings of Vishakhapatnam.

Preparation of methanolic and aqueous Extracts

The air dried and powdered fruits of *Momordica cochinchinensis* (1.5kg) were extracted in soxhlet with aqueous and methanolic (2 each) and subsequently was concentrated to a small volume. The concentrated extracts were tested for anti-microbial activity.

Identification of the plant constituents by phytochemical tests [12, 13]

Ethanolic extract is subjected to various preliminary phytochemical analysis to test for the presence or absence of various phytoconstituents by the following tests.

1. Test for alkaloids:

To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloid reagents

a) Mayer's test:

The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

b) Dragendroff's test:

The filtrate will be treated with Dragendroff's reagent: appearance of reddish brown precipitate indicates the presence of alkaloids.

c) Hager's test:

The filtrate when treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

2) Test for carbohydrates and reducing sugar

The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to

a) Molisch's test:

A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

b) Fehling's test:

The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

c) Benedict's test:

The extract will be treated with Benedict's reagent; appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

d) Barfoed's test:

The extract will be treated with barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non reducing sugars.

3) Test for steroids:**Liebermann bur chard's test:**

The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins:**a) Biuret test:**

The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.

b) Millon's test:

The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5) Test for tannins:

The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds:

a) The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.

b) The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids:

a) 5ml of extract will be hydrolyzed with 10% sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids.

b) Shinoda's test:

The extract will be dissolved in alcohol, to which few magnesium turnings will be added followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8. Test for gums and mucilage:

The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will be examined for its swelling properties.

9. Test for glycosides

When a pinch of the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of a ring at the junction of two liquids indicates the presence of glycosides.

10. Test for saponins

Foam test

About 1 ml of the extract was diluted to 20 ml of with distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11. Test for Triterpenoids

The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids.

Antioxidant activity by DPPH method [11]

DPPH free radical scavenging activity

The free radical scavenging activity will be measured *in vitro* by 1, 1-diphenyl-2-picryl-hydrazyl assay. About 0.3 mM solution of DPPH in 100% ethanol will be prepared and 1ml of this solution will be added to 3ml of the extract dissolved in ethanol at different concentrations (5-80mcg/ml).The mixture has to be shaken and allowed to stand at room temperature for 30 min .Absorbance will be measured at 517 nm using a spectrophotometer.The capability to scavenge the DPPH radicals will be calculated using the formula.[11]

$$(\%) \text{ scavenged} = \frac{(\text{A control}-\text{A test})}{\text{A control}} \times 100$$

A control is the absorbance of the control reaction mixture.

A test is the absorbance of sample of the extract at different concentrations

Determination of antimicrobial activity [10]

Fresh aqueous and methanolic extracts of *Momordica cochinchinensis* were used for the determination of antimicrobial activity.

Microorganism used

Staphalococcus aureus (gram+ve)

Escherichia coli (gram-ve)

Standard drug

Ampicillin

Method

Agar well diffusion method

From the above mentioned organisms, inoculums was prepared by inoculating the organisms in 10 ml of nutrient broth and incubated at 37 degrees centigrade for 18 hrs. Nutrient agar medium was poured in to each sterilized petridish and organism was inoculated. Wells were made in to the medium by using sterile cork borer and each sample of the extracts (100ul-500μl) was filled in to the wells of agar plates directly by using a micro liter syringe .Then the plates were incubated at 37 degrees centigrade for 24 hr.After incubation, the zone of inhibition was observed and measured in mm. [10]

RESULTS**Preliminary phytochemical screening for methanolic extract of *Momordica Cochinchinensis***

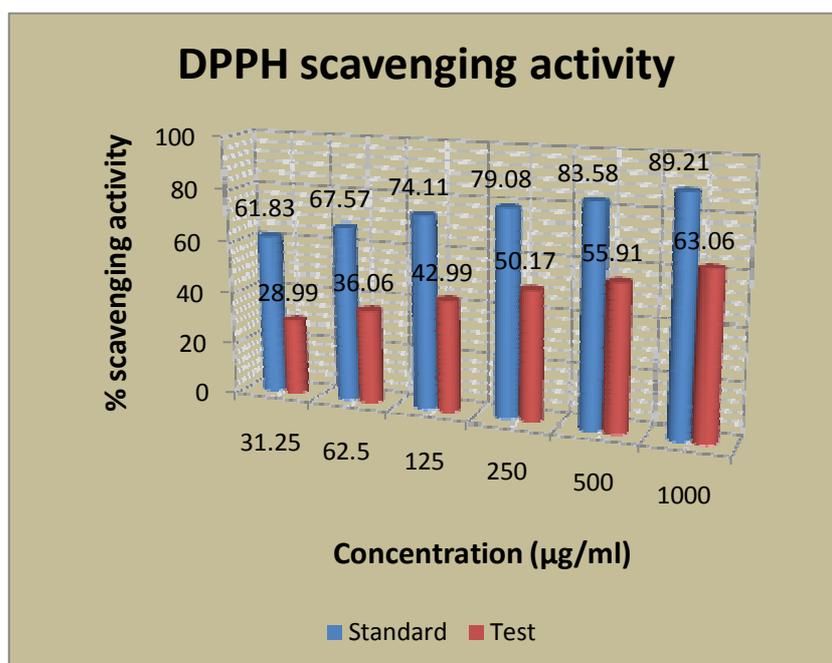
S.NO	CONSTITUENTS	OBSERVATIONS
1	phytosterols	+
2	Glycosides	+
3	Carbohydrates	+
4	Flavanoids	+
5	Alkaloids	-
6	Tannins	-
7	Proteins	-
8	Saponins	-
9	Gums and mucilages	-
10	Phenolic compounds	-
11	Steroids	-

+ = Present

- = Absent

Antioxidant values by DPPH method

S.NO	CONCENTRATION ($\mu\text{g/ml}$)	%INHIBITION FOR TEST DRUG	%INHIBITION FOR STANDARD DRUG
1	31.25	28.99	61.83
2	62.5	36.06	67.57
3	125	42.99	74.11
4	250	50.17	79.08
5	500	55.91	83.58
6	1000	63.06	89.21



Antimicrobial activity for (gram + ve) *Staphalococcus Aureus*

S.NO	TEST CONCENTRATION ($\mu\text{g/ml}$)		STANDARD CONCENTRATION ($\mu\text{g/ml}$)	ZONE OF INHIBITION (mm)		
	Aqueous	Methanol		Aqueous	Methanol	Standard
1	100	100	100	10	15	42
2	200	200	200	12	18	54
3	300	300	300	15	22	62
4	400	400	400	17	26	74
5	500	500	500	18	29	87

Antimicrobial activity for (gram -ve) *Escherichia Coli*

S.NO	TEST CONCENTRATION ($\mu\text{g/ml}$)		STANDARD CONCENTRATION Ampicillin ($\mu\text{g/ml}$)	ZONE OF INHIBITION (mm)		
	Aqueous	Methanol		Aqueous	Methanol	Standard
1	100	100	100	11	14	44
2	200	200	200	14	17	57
3	300	300	300	16	19	66
4	400	400	400	19	22	78
5	500	500	500	22	25	90

DISCUSSION

Preliminary phytochemical studies were performed for methanolic extract of *Momordica cochinchinensis*. The results indicate the presence of glycosides, flavonoids, carbohydrates and phytosterols. Antioxidant activity by DPPH method shows better results for test drug when compared to the standard drug (ascorbic acid). In both cases as concentration increases, the % of inhibition also increases for antioxidant activity. Antimicrobial activity was screened by using agar well diffusion method. The results revealed that the methanolic extract exhibited significant anti-microbial activity at concentration of 100-500 $\mu\text{g/ml}$ respectively against tested organisms, particularly more effective against *gram (+)ve* bacteria *Staphalococcus aureus*, and *gram (-) ve* bacteria *Escherichia coli*. than the aqueous extract when compared to the standard drug (Ampicillin).

CONCLUSION

The tested bacteria significant susceptibility to the methanolic extract and aqueous extracts of *Momordica cochinchinensis* fruits two of the tested bacteria *staphalococcus aureus*, (*gram+ve*), *Escherichia coli* (*gram-ve*) were found to be more sensitive to methanolic and aqueous extracts of *Momordica cochinchinensis* fruits, further revealed that the amount of activities increases with concentration of extract. The results indicate that the methanolic extract has shown more degree of anti-microbial activity than the aqueous extract when compared to the standard drug (Ampicillin). It shows good antioxidant property for test drug when compared to the standard drug. It is due to the presence of chemical constituents like carbohydrates, flavanoids and glycosides which was conformed by phytochemical studies.

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