

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF WHOLE PLANT OF *Madhuca indica*

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ABSTRACT

The methanolic extract of *madhuca indica* was subjected to preliminary phytochemical studies. The results include indicate the presence of alkaloids, proteins, carbohydrates. anti microbial activity of methanolic extract and aqueous extracts of *madhuca indicia* was screened by agar well diffusion method. The results revealed that the methanolic extract exhibited significant antimicrobial activity of concentration of 100-500 µg/ml respectively against tested organisms, particularly more effective against gram – ve bacteria *staphylococcus aureus* and gram –ve bacteria *Escherichia coli* than the aqueous extract when compared to the standard drug (ampicillin).

Keywords: *Madhuca indica*, antimicrobial activity, physicochemical studies.

INTRODUCTION

Madhuca indica commonly known as butter tree which belongs to family *sapotaecae* and it is distributed in north Indian plains and in forests of kerala.karnataka,madya Pradesh and Rajasthan. it is commonly available in wastelands, by arable lands and plaeotropic lands. generally the calyx contain 5 lobes. upper lip ovate, lower lip sericeous without villous within.

Te corolla is white in colour and contains 5 lobes and they are in acute shape. they are having 4 stamens. The seeds are mucilaginous in nature. the main chemical constituents in *madhuca indica* are tannins, saponins and steroids,β-amyirin, β -amyirin acetate, β -amyirin cinamate, β -amyirin decanate, betullic acid, ursolic acid, stigma sterol,- β carotene, qnercetin are present. The main uses of *madhuca indica* are carminative, demulcents, emollient, laxative, astringent and tonic. The leaves of *madhuca indica* are used in treatment of eczema and seeds are used to relive pain in muscle and in joints. The flowers are used in treatment of cough and bronchiolitis and for making vinegar and liquor. The bark is used for treatment of ulcers and wounds. The external applications are skin

effections, analgesic anti pyretic,anti-oxidant and anti diabetic.

MATERIALS AND METHODS

EXPERIMENTAL SECTION

Normally the butter tree grows in the forests of Karnataka, Kerala, Madhya Pradesh, Andhra Pradesh, and Rajastan.The plants were collected in December 2011 from the surroundings of Kurnool. It is commonly distributed in gregarious waste lands, by arable lands and plaeotropic.

PREPARATION OF METHANOLIC EXTRACT AND AQEOUS EXTRACT

The air dried and powdered parts of *madhuca indica* (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.

PREPARATION OF TEST AND STANDARD SOLUTIONS

The stock solution of test compounds was prepared by dissolving the dried extracts at a concentration of 1mg/ml and pure compounds

at a concentration of 1mg/ml in di-methyl sulphoxide (DSMO) respectively. The stock solution of reference standards (*ampicillin*) was prepared at a conc. of 100µg/ml in sterile water. Anti-microbial activity was screened by adding 100-500µg/ml of test and standard solutions to each cup by micropipettes.

DETERMINATION OF ZONE OF INHIBITION BY CUP-PLATE METHOD

The anti-microbial activity in terms of zone of inhibition for methanolic and aqueous extract of *madhucaindica* was determined against 2 micrograms and the results were compared with *ampicillin* as standard. All the dilutions for the preparation of test (100-500µg/ml) and standard (100-500µg/ml) drug were prepared, the inoculum was spread on the surface using a sterile cotton swab and wells were made by using sterile cork bore of 6mm diameter each. Then the cups were filled accordingly. After introducing the sample, standard and control in the cups the Petri plates were kept in refrigerator at 4°C for 2hrs, for diffusion and then incubated at 37°C for 24hrs. The anti-microbial activity was measured as a diameter in mm of inhibitory zones on the agar plates. The experiment was repeated in triplicate and the average value was written.

The methanolic and aqueous extracts of *madhucaindica* plant powder were screened for anti-microbial activity against a wide spectrum of microorganisms and the activity was compared with appropriate reference standards (*ampicillin* for both *gram +ve* and *gram-ve* organisms) microorganisms were grown in nutrient agar medium. Dimethyl sulphoxide was used as the plant extract and reference standards respectively.

MEASURING THE ZONE OF INHIBITION

The presence of definite zone of inhibition of any size around the cup indicated anti-microbial activity. The solvent DSMO was run simultaneously to assess the activity of test drug, which was used as a vehicle. The results were mentioned in the activity of test drug, which was used as a vehicle. The results were mentioned in table: 3 and 4.

CHEMICAL TESTS

Identification of Plants Constituents By Phytochemical Tests

Ethanol extract is subjected to various preliminary phytochemical analysis to test for the presence or absence of various phytochemical constituents by the following

tests. The results were mentioned in table 1, 2.

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) Dragon druff's test

The filtrate will be treated with dragon druff's reagent: appearance of reddish brown colour precipitate indicates the presence of alkaloids

C) Hager's test

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

2) Test for carbohydrates and reducing sugars

A) Molish's test

To a small portion of filtrate add a little amount of molish's reagent and H_2SO_4 : formation of a violet ring indicates the presence of carbohydrates.

B) Fehling's test

To a small portion of filtrate add a little amount of Fehling's reagent A and Fehling's reagent B: formation of a reddish brown indicates the presence of carbohydrates

C) Benedict's test

To a small portion of filtrate add a little amount of benedict's reagent: formation of a reddish orange indicates the presence of carbohydrates.

D) Barfoeds test

To a small portion of filtrate add a little amount of barfode's reagent: formation of a reddish orange indicates the presence of carbohydrates.

3) Test for steroids

Libbermann 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 H_2SO_4 .

4) Test for proteins**A) Biuret test**

To the extract add small amount of copper sulphate solution followed by addition of NaOH solution. Formation of a violet colour indicates the presence of proteins.

B) Million's test

To a small portion of filtrate add a little amount of million's reagent: formation of a pink colour indicates the presence of proteins.

5) Test for tannins

The extract will be treated with 10% lead acetate solution. Formation of a white colour indicates the presence of tannins.

6) Test for phenolic compounds

A) Extract treated with neutral ferric chloride: formation of a violet colour indicates the presence of phenolic compounds.

B) Extract will be treated with 10% NaCl solution formation of a cream colour indicates the presence of phenolic compounds.

7) Test for flavinoids

A) 5ml of extract will be hydrolyzed with 10% of H_2SO_4 and cooled. then it will be extracting with diethyl ether and divided into three portions in 3 separate test tubes, 1ml of diluted sodium carbonate, 1ml of 0.1 N sodium hydroxide and 1ml of strong ammonia solution will be added to the first, second and third test tube respectively in each test tube. Development of yellow colour demonstrated the presence of flavinoids.

B) Shinoda's test

The extract will be dissolved in alcohol. To which few magnesium turnings will be added followed by concentration HCl drop wise and heated and appearance of magenta colour indicates the presence of flavinoids.

8) Test for gums and mucilage

To the extract add 25ml of absolute alcohol and filtered. The filtrate was 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 addition of concentrated H_2SO_4 , formation of a ring at junction of two liquids.

10) Test for saponins**Foam test**

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

11) Test for triterpenoids

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

DISCUSSION

Preliminary phytochemical studies were performed for methanolic extract of *Madhuca indica*. the results indicate the presence of alkaloids, tannins, proteins, and carbohydrates. anti microbial activity was screened by using agar well method. the results revealed the methanolic extract exhibited significant anti microbial activity of conc of 100-500(mg/ml) respectively against tested organism particularly more effective against gram +ve bacteria *Staphylococcus* 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 *Madhuca indica* two of tested bacteria *Staphylococcus* gram +ve and *Escherichia coli* gram -ve were to be more sensitive to methanolic and aqueous extract of *Madhuca indica*. Further revealed that the amount of activity increased with the concentration of extract. The results indicate that the methanolic extract has shown more degree of anti microbial activity than aqueous extract when compared to the standard drug (ampicillin). it shows anti microbial activity when compared to standard drug. it is due to presence of chemical constituents like carbohydrates, flavinoids, and tannins proteins which was confirmed by phytochemical studies.

Table 1:

Physico chemical parameters of <i>madhucaindica</i>		
S.No	Parameters	Values (%)
1	Total ash values (%)	0.18%
2	Water soluble ash (%)	0.08%
3	water soluble extractive(%w/w)	0.66%
4	alcohol soluble extractive(%w/w)	0.68%
5	Loss on drying(%)	18%

Table 2:

preliminary phytochemical screening of <i>madhucaindica</i>		
S.No	constitutes	observations
1	Alkaloids	+
2	Tannins	+
3	Proteins	+
4	Flavonoids	-
5	carbohydrates	+
6	amino acids	-
7	volatile oils	-

Table 3: Anti microbial activity of *madhuca indica*.
Staphylococcus aureus (gram+ve)

S. No	Test concentration		Standard concentration ($\mu\text{g/ml}$)ampicillin	Zone of inhibition(mm)		
	aqueous	methanolic		aqueous	methanolic	Standard
1	100	100	100	11	17	18
2	200	200	200	13	20	21
3	300	300	300	16	24	25
4	400	400	400	18	27	29
5	500	500	500	19	29	34

Table 4: Anti microbial activity of *madhuca indica*
Escherichia coli (gram-ve)

S.No	Test concentration		Standard concentration($\mu\text{g/ml}$)	Zone of inhibition(mm)		
	aqueous	methanolic		Aqueous	Methanolic	Standard
1	100	100	100	12	15	16
2	200	200	200	16	19	20
3	300	300	300	18	21	22
4	400	400	400	20	22	27
5	500	500	500	23	27	30

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