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Extraction and analysis of mango leaf components and testing their anti diabetic potential

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Abstract
Diabetes is one of the life threatening metabolic disorders that has no cure but only management. Various therapeutic agents are available in nature which can aid in blood sugar management. However role of Mango in diabetes was always known to be negative and thought to increase blood sugar levels. Objective of the study is to test the therapeutic role of Mango leaf extract in diabetic control. The anti diabetic effect of leaf extract was studied based on the principle of alpha glucosidase enzyme inhibition test. Further, secondary metabolites present in leaf extract were analyzed biochemically. Antimicrobial potential of the extract was studied against 4 test bacteria which included *Escherichia coli, Enterobacter spp, Pseudomonas aeroginosa and Staphylococcus aureus*. Kirby bauer's method of disc diffusion was used for this assay. All the results of the study were analyzed to confirm the therapeutic potential of mango leaves.

Keywords: Mangifera indica, Antimicrobial, Antidiabetic, Discdiffusion, Zone of Inhibition.

Introduction

Mango Tree, a magical tree of India scientifically called as *Mangifera indica* belongs to the family Anacardiaceae [9], is an annual flowering plants with yearly fruit production. Mango being one of the delicious fruits known internationally, it also has a wide rage of applicability. All parts of the tree like, leaves, bark, fruits etc have different uses [5]. According to the ancient Indian culture and tradition Mango leaves has a special importance in every festivals, poojas and celebrations[8]. The leaves are used in decorating entrance of a home and door. They are also used in kalusha for prayers, fruits are used in prasadas. Sacred Ramayana also mentioned the impatience of Aamra in its various chapters.

Apart from its regular traditional importance mango tree also has a key role in medicine and therapy [1]. It was termed Aamra in different vedic scripts and has been used in aryurvedic medicine system since 4000years [7]. Some of the medicinal properties of mango tree include antioxidant, anticancer, antimicrobial, anti inflammatory, radio protective, anti allergic, anti modulatory and surprisingly it also posses anti diabetic properties [2]. It is a wonderful source of many polyphenolic compounds in particular it posses magiferin with potential therapeutic role [6]. It can be obtained from all parts of the tree and is a super antioxidant which is known to be xanthone derivative [4].
An adverse effect of mango fruit and its juice on blood sugar levels is known since long. However, it is surprising to know that the extracts of leaf and stem are known to posses anti diabetic activity and show a positive influence on blood sugar levels. Chlorogenic acid and chicoric acid are the two known vital photochemicals involved in elevating the glucose intake by cells thereby having a positive influence on blood sugar levels [3]. Most of the studies in diabetic research are conducted in rodents especially rats to detect the role of compounds in blood sugar levels. The studies revealed a potential anti diabetic effect of plant extract by mechanisms like increased glucose tolerance, improved lipid profile etc. An intense research in his aspect of Mango plant can be encouraged to expect a future generation with diabetics free population.

Materials and Methods

Preparation of Leaf extract

Mangifera indica leaves were collected from the garden in BioAxis DNA Research Centre, Hayath nagar Hyderabad. All the leaves were washed with distilled water and were subjected for oven dry at 50^0C, ensuring that the temperature is regulated and will not harm the phytochemicals of the leaf. The dried leaves were homogenized and ground into very fine powder in a motor pestle and the leaf extract was prepared using methanol as a solvent using soxhlet extractor. The extract thus obtained was

filtered using whatman nuber1 filter paper. The extract was subjected for drying in a rotary evaporator. The extract can be stored at -20^{0}C.

Disc diffusion to test the antimicrobial activity of extract

Initially a basic level analysis was performed to analyze the inhibitory effect of extract on microbial pathogens. The prepared leaf extract was measured and the standard concentration were used to prepares the discs by immersing them blank discs of whatman paper in extract. Different concentrations of the discs ranging in 50, 100, 200 and 400mg are prepared. Kirby Bauer's standard disc diffusion method was used to analyze the antimicrobail activity of the extract. Pathogenic bacteria selected for the study were *Escherichia coli*, *Enterobacter spp*, *Pseudomonas aeroginosa and Staphylococcus aureus* which are some of the common human pathogens. Pure cultures were obtained from certified stocks of Bioaxis DNA Research Centre. The ultures were activated by thawing followed by sub culturing on Nutrient agar media followed by heir used in disc diffusion method.

Isolation of various phytochemicals from leaf extract

Various phytochemicals to be tested in extract include steroids, cardiac glycosides, saponins, tannins, flavonoids and alkaloids.

Detection of steroids in the extract

Lieberman Burchard method was used for this test: To 1ml of the extract 5ml of anhydrous acetic acid is added and mixed. The above mixture is treated with conc sulphuric acid . The color of the solution changes from pink to red, violet, blue or green depending on he type of compounds present.

Keller Killians test for the presence of cardiac glycosides

To the leaf extract 1ml of glacial acetic acid and a drop of ferric chloride solution. To the above solution 1ml of conc sulphonic acid. Formation of a brown colored ring at the junction of two layers is an indication for he presence of ade-oxy sugars which is a characteristic property of carotenoids.

Test for saponins

To 1m of extract add 1 to 2ml of water and shack thoroughly for few seconds. Formation of froth is an indication for the presence of saponins.

Identification test for tannins

To the extract 1ml, few drops of alcoholic FeCl3 0.1% is added. Formation of dark green to black or blue color formation indicates the presence of tannins.

Flavonoids identification test

To 1ml of the extract 0.5ml of 10% NaOH is added. The color of the solution changes to yellow. On the addition of dilute hydrochloric acid yellow color disappears indicating the presence of flavonoids.

Test for alkaloids

To 1ml of extract 1ml of mayer's reagent is added. Formation of white precipitate is an indication for the presence of alkaloids.

Anti diabetic activity based on enzyme inhibition

Testing the anti diabetic efficiency of extract is based on the inhibition of alpha glucosidase enzyme of yeast. The test can be performed using PNPG (para nitro phenyl alpha D glucopyranoside) as the key substrate. In this test 96 well microtitre plate can be used to which 30micro litre of standard enzyme of the concenration 0.5U/ml should be added. To this enzyme 30micro litre of sodium phosphate buffer of 0.1M concentration is o be added and 30microlitre of test extract from Sigma Aldrich, USA in DMSO should be also added. The solutions can be mixed and must be incubated for 10 min at room temperature.Later to the same well 30microlitre of substrate must be added and re incubated at room temperature for 20min. In order to terminate the reaction 80micro litres of 0.2micro molar sodium carbonate is to be added. The OD of the solution can be read at 405nm using microplate reader. All the readings must be taken in triplicates in increase the accuracy.The inhibition of the enzyme was calculated in % using the formula:

$$\%\text{Inhibiion of enzyme} = \frac{\text{OD OF CONTROL- OD OF EXTRACT}}{\text{OD OF CONTROL}} * 100$$

Results and Discussion

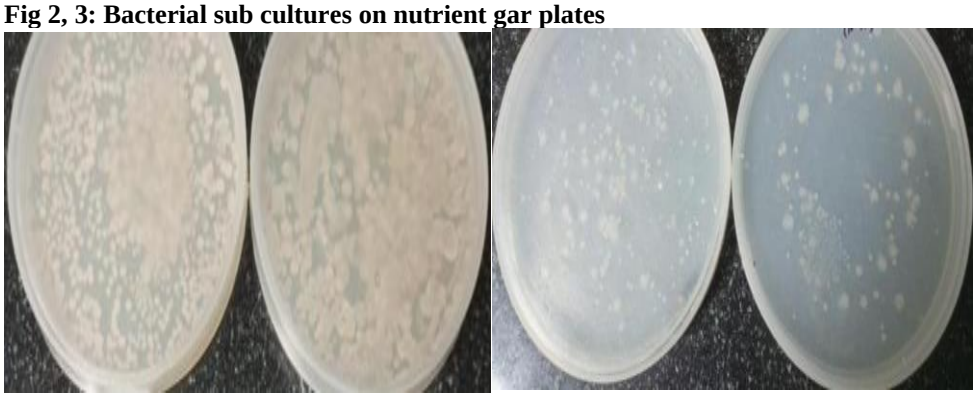
Extract preparation from leaves: After collecting the leaves and drying the powdered leaf was used for extraction with ethanol followed by its filtration. Fig 1 shows the extract being filtered using whatman no 1 filter paper.

Fig1: Filtration of extract

Inference: The above figures shows the collection of extract by filtration using whatman no 1 filter paper.

Once the extract is ready t be tested all the bacterial stocks were sub cultured on to Nutrient agar plates. Fig 2 shows the bacterial subcultures used for testing the inhibitory effect of plant extract.

Fig 2, 3: Bacterial sub cultures on nutrient gar plates



Inference: The above picture shows some of the plates of sub cultures showing bacterial colonies based on the initial cell count the no of bacterial colonies in the plates vary as shown above.

The extract obtained was tested for its inhibitory effect on different isolated cultures and the zones obtained were observed.

Fig 4: Zones of inhibition against the test bacteria

Inference: One of the plate showing zones of inhibition with varied concentrations of extract and 1 control disc. Such test was performed for all the bacteria

All the zones obtained with different concentrations of extract against different bacteria were measured and summarized below.

Table 1: Zones of inhibitions obtained for different concentration of extract against bacteria

SNO	Bacterial Species	Length of zone with conc 50mg/ml of extract	Length of zone with conc 100mg/ml of extract	Length of zone with conc 200mg/ml of extract	Length of zone with conc 400mg/ml of extract
1	*Escherichia coli*	10mm	14mm	17mm	21mm
2	*Enterobacter spp*	12mm	15mm	16mm	22mm
3	*Pseudomonas aeroginosa*	8mm	12mm	16mm	18mm
4	*Staphylococcus aureus*	5mm	7mm	12mm	15mm

Inference: The above table shows various ranges of zone of inhibitions obtained for different concentrations of leaf extracts against different bacteria. Highest inhibition was observed for both *Escherichia coli* and *Enterobacter spp.* With the increase in the concentration of the extract he size of zone obtained was also increasing indicating a direct relation between the Zone length and concentration used.

In addition to the inhibition assay a genera phytochemical analysis of the extracts was conducted whose results are furnished below in Table 2

Table 2: Phytochemical components in leaf extract:

S.NO	TEST	RESULT
1	Cardiac Glycosides	Present
2	Saponins	Absent
3	Tannins	Present
4	Alkaloids	Present
5	Flavonoids	Present
6	Steroids	Present

Inference: The above table depicts all the phytochemicals present in the leaf extract of moringa

Conclusion

In the current work biochemical analysis of mango leaves was performed especially targeting its anti diabetic effect. For this some mango leaves were collected whose extract was prepared following standard protocols. The extract was subjected for phytochemical analysis as specified in the procedure. The results indicated that the methanol extract of leaves contained Steroids, Flavanoids, alkaloids, cardiac glycosides except saponins. The antimicrobial analysis of the extract revealed its great effect against all the test bacteria with the highest inhibition against both *E. coli and Enterobacter.* To detect the antidiabetic role of extract a colorimetric assay was selected which is based on the inhibition of alpha glucosidase. The results of the study revealed a considerable role of extract in inhibiting the enzyme which is an indication for its anti diabetic effect. Thus the study supports the ability of mangifera leaves in controlling blood sugar levels if used regularly for extract consumption.

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