

Pharmacological studies of *Mangifera indica* leaf extract

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World Journal of Biology Pharmacy and Health Sciences, 2021, 07(03), 073–079

Publication history: Received on 14 August; revised on 24 September 2021; accepted on 26 September 2021

Article DOI: <https://doi.org/10.30574/wjbphs.2021.7.3.0100>

Abstract

The present study was undertaken to explore the phytochemical screening, anti-bacterial and anti-oxidant activities of the hydro-methanolic leaves extract of *Mangifera indica* using standard screening methods such as disc diffusion and DPPH methods. In phytochemical screening, *Mangifera indica* extract showed presence of secondary metabolites such as carbohydrate, phenols, tanins and proteins whereas Saponins were absent. It also showed antibacterial activities against almost all the test organisms. The extracts possessed potent hydroxyl radical scavenging activity against the positive control standard Ascorbic acid. Results denote the presence of hydroxyl radical scavenging principles in the extracts.

Keywords: Antibacterial; Antioxidant; Phytochemical; *Mangifera indica*

1. Introduction

Plants and their secondary metabolite constituents have a long history of use in modern western medicine and in certain systems of traditional medicine and are the sources of important drugs such as atropine, codeine, digoxine, morphine, quinine and vincristine etc. Use of herbal medicine in developed countries has expanded sharply in the latter half of twentieth century. The pharmacological treatment of disease began long ago with the use of herb

Mango (*Mangifera indica* L.) is a juicy stone fruit belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. The unripe fruits are acidic, acrid, antiscorbutic, refrigerant, digestive and carminative. They are useful in dysentery ophthalmia, eruptions, urethrorrhoea and vaginopathy. The ripe fruits are refrigerant, sweet, emollient, laxative, cardioprotective, haemostatic, aphrodisiac, and tonic. They are also used in vitiated conditions vata and pitta, anorexia, dyspepsia, cardiopathy, haemoptysis, haemorrhages from uterus, lungs and intestine, emaciation, and anemia.

As reported earlier, about 74% of the known anti-cancer medicines are derived from various plant species [1,2]. Indeed, there are many households dietary products exhibiting anti-cancer potential with minimal side effect that are currently under clinical trials for cancer treatment [3,4]. Among these, two important household dietary products that are very common among South Asian communities are Curcumin and Lycopene. Curcumin is a polyphenolic compound isolated from turmeric and this product exhibits anti-microbial, immunomodulatory, and potential cancer chemo preventive efficacy [5,6]. Lycopene is a carotenoid compound abundant in tomatoes and also present in tomato products [7]. As reported earlier lycopene and its derivative exhibit anti-cancer properties. In addition to this the product can also be used in the treatment of cardiovascular diseases [8]. Noratto et al., (2010) compared the anticancer properties of polyphenolic extracts from several mango varieties in cancer cells lines, including Molt-4 leukemia, A- 549 lung, MDA-MB-231 breast, LnCap prostate, SW-480 colon cancer cells and non-cancer colon cell line CCD-18Co [9]. Ali et al., (2012)

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and Timsina et al., (2015) determined that ethanol extract had significant cytotoxicity to HeLa cells and the bioactive fraction from the crude extract had antiproliferative effects with an IC₅₀ value of <10 µg/ml [10-11]. Bhowmik et al., (2009) found that Single oral administration of a dose of 250 mg/ kg body weight produces a potent and strong hypoglycemic effect in Type-2 diabetes on rats. Similar result was found by Reda MY, (2010) [12]. Beltrana AE et al., (2004) reported that anti- inflammatory action of mangiferin is related with the inhibition of NOS and cyclooxygenase-2 expression [13]. The possible anti-inflammatory mechanisms of mangiferin include the balance between the overwhelming anti-inflammatory cytokines and proinflammatory mediators, inhibition of inflammatory cellular activations, regulations of inflammatory gene expressions, and enhancements of the cellular resistance against inflammatory injuries [14-16]. Chemopreventive properties of mango pulp extract (MPE) was evaluated in alteration in liver of Swiss albino mice. MPE was found to be effective in combating oxidative stress induced cellular injury of mouse liver by modulating cell-growth regulators [17]. The aqueous and ethanol extract of leaves and stems of mango at 50 and 25 mg/ml has been found sufficient activity against bacteria; *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Enterococcus faecalis* [18]. It is thought that the antibacterial activity of mango extract is due to the presence of gallotannin and mangiferin [19]. The present study carried out on the investigation of phytochemicals screening, anti-oxidant and anti-bacterial activities using *in vitro* model.

2. Material and methods

2.1. Plant collection and identification

The leaves of *Mangifera indica* were procured from Satna (Madhya Pradesh) and authenticated by Botanist, Dr. Manoj tripathi of DRI, Chitrkoot Madhya Pradesh (India).

2.2. Chemicals

All the materials and reagents used for the study were from CDH, Renchem and Hi- Media Ltd., India.

2.2.1. Preparation of *Mangifera indica* leaves extract

The collected seeds were dried in shade and grinded with mechanical grinder. About 50g powder poured in separating funnel with 50% methanol for 48hrs. The collected residues were kept at 55-60°C in water bath to concentrate it and finally transfer into the Hot Air Oven to dry it. About 5.8g crude extract was prepared (Yield= 19%) and used for the further studies.

2.3. Phytochemical screening

Phytochemicals are nonnutritive plant chemicals that contain protective, disease- preventing compounds. Standard screening test were carried out for various plant constituents. Hydro-methanolic crude extract were screened for presence or absence of secondary metabolites such as alkaloids, tannins, steroids, phenols, flavonoids, saponins and Phlobatannins etc. using standard procedures to identify the constituents as described by Sofowara, Trease and Evans, Harborne.

2.3.1. Test for Carbohydrates

A small quantity of extract was dissolved in 4 ml of double distilled water and filtered. The filtrate was subjected to molisch's test to detect the presence of carbohydrates and further addition of Fehling's reagent. It showed the brick red color confirmed the presence of reducing sugar.

2.3.2. Test for Proteins

About 2ml of filtrate was treated with 2ml of 10% sodium hydroxide solution in a test tube and heated for 10 minutes. A drop of 7% copper sulphate solution was added in the above mixture. Formation of purplish violet color indicates presence of proteins.

2.3.3. Test for Glycosides (Keller-Killani test)

A portion of the hydro-methanolic plant extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish-brown coloration at the junction of two layers and the bluish green color in the upper layer showed presence of glycosides.

2.3.4. Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample.

2.3.5. Test for Free Flavonoids

A portion of the powdered plant sample was heated with 10ml of ethyl acetate over a steam bath for 3min. the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonium solution. A yellow coloration was observed indicating a positive test for flavonoids.

2.3.6. Lead acetate test

0.5gm plant extract was heated with double distilled water followed by addition of 1ml of 10% lead acetate solution. The yellow precipitate indicates the presence of flavonoids.

2.3.7. Reaction with Sodium hydroxide

0.5gm plant sample was heated with double distilled water followed by addition of dilute sodium hydroxide. The yellow color indicates presence of flavonoid.

2.3.8. Test for alkaloids Preliminary test

100mg of plant extract was dissolve in dilute Hcl afterward the solution was filtered, filtrate was tested with Dragendroff's and Mayer's reagents.

3. Anti-bacterial activities

Antibacterial activities of hydro-methanolic extract from leaves of *Mangifera indica* was investigated using the Disk diffusion method given by Kerby-Bauer Disk Diffusion Susceptibility test.

3.1. Bacterial strain

Following gram negative and gram-positive bacterial strain i.e., *Escherichia coli*, and *Bacillus subtilis* used for the Antibacterial activities which were received from stock culture of our laboratory.

3.2. Media

Nutrient agar broth media were used for the antibacterial activities. Nutrient broth is prepared i.e. 1.3g in 100ml of double distilled water, poured indifferent test-tubes and added bacterial strain in each test-tube. Nutrient Agar media prepared poured in Petri- plates after solidifying swab the bacterial cultures on the plates and allowed for incubation at 37°C for 24 hrs.

3.3. Concentration

4 different concentrations of crude extract were prepared (100%, 75%, 50%, 25%). 100%= 1g crude extract in 1ml of double distilled water freshly prepared afterward serial dilution prepared 75 %= 75mg in 1ml, 50 %= 50mg in 1ml, 25 %= 25mg in 1ml.

3.4. Study parameter

Measurement of Zone of Inhibition (In mm).

3.5. Anti-oxidant activities

Anti-oxidant activities of *Mangifera indica* leaves extract (10-100 µg/ml) were determined according to standard DPPH methods.

4. Results and discussion

4.1. Phytochemical screening

The phytochemical present in the hydro methanolic extract of *Mangifera indica* leaves extract are carbohydrate, Phenol, Tanins, Protein whereas Saponin were absent (Table 1)

Table 1 Phytochemical present in the hydromethanolic extract of *Mangifera indica* leaves extract

S.No.	Phytochemical Test	Hydromethanolic Extract
I	Test for Carbohydrates and reducing sugars	
A	Fehling's Test	+
II	Test for Phenolic compound's	
A	Ferric Chloride test	+
III	Test for Tannins	
A	Lead Acetatetest	+
IV	Test for Phytosterols	
A	Salkowski Test	-
V	Test for Proteins	
A	Biuret Test	+
VI	Test for Saponins	
A	Foam Test	-
VII	Test for Flavanoid	
A	Lead AcetateTest	-

4.2. Antibacterial Activity

Table 2 Antibacterial activity of *Mangifera indica* leaves against bacterial strains

Name of microorganisms	% Concentration of Extract [zone of inhibition(mm)]			
	25	50	75	100
<i>B. subtilis</i>	18	19	20	21
<i>E. coli</i>	20	20	21	23

100 % - 1 gm / 1 ml/D.W., 75 % - 750mg/1ml /D.W, 50 % - 500mg/1ml/D.W - 25% - 250 mg/1ml/D.W

4.3. Antioxidant Activity

The *in vitro* antioxidant activity of *Mangifera indica* leaves extract was tested in various concentrations against Ascorbic acid as standard.

Table 3 *In vitro* antioxidant activity of 50% methanolic *Mangifera indica* extracts Vs Ascorbic acid (standard)

Sr. No.	Concentration of ascorbic acid (µg)	% TBARS inhibition	Concentration of <i>Mangifera indica</i> (µg)	% TBARS inhibition
1	100	31.18	100	12.97
2	200	36.65	200	14.08
3	300	37.42	300	12.47
4	400	47.89	400	37.72
5	500	57.54	500	42.35
6	600	16.70	600	51.10
7	70	61.87	700	51.10
8	80	71.30	800	10.96
9	90	72.27	900	13.58

Percentage of TBARS was calculated for both Ascorbic acid and *Mangifera indica* extract, with the help of formula, for a comparative study. The percentage of TBARS was plotted in the graph in different concentration as per following formula.

$$\frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

5. Discussion and Conclusion

Plants are one of the most important sources of medicines. The role of medicinal plants in promoting the ability of human health to cope with the unpleasant and difficult situations is well documented from ancient times till date all over the world. One of the cardinal goals is the quest to combat the incidence of diseases such as malaria, HIV/AIDS and chronic diseases such as age-related degenerative diseases, cancer and cardiovascular diseases. Medicinal plants are rich in secondary metabolites which are potential sources of drugs and of therapeutic importance. There is increasing interest in the use of plant extracts as therapeutic agents. Mango 'king of fruit' belongs to use pharmacological potential as panacea. It is reported that mango is a potential source of anticancer, anti-diabetic, anti-inflammatory, source of anticancer, anti-diabetic, anti-inflammatory, antimicrobial drugs as well as it also used as cardio protective, radio protective, recognition of memory and many others. Our result suggest the antibacterial and antioxidant properties if *Mangifera indica* leaves extract which is important for further study for anticancer and antidiabetic activity of this plant.

Compliance with ethical standards

Acknowledgments

The authors are thankful to our dissertation students Ms. Vaishali Singh and Ms. Dahiya for carrying out the above work and Dr. S.K. Maheshwari, Medical Director of M. P. Birla Hospital, Satna for providing lab facilities to carry out above work.

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