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***In-vitro* analysis of antioxidant and antimicrobial properties of *Garcinia mangostana* L. (pericarp) and *Clitoria ternatea* (flower)**

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Abstract

The present study was to evaluate the phytochemical analysis, antimicrobial activity and radical scavenging and antioxidant properties of the *Garcinia mangostana* L. pericarp and *Clitoria ternatea* flower (blue) extract obtained by methanol solvent. The extracts were evaluated antioxidant activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) and antimicrobial activity through well diffusion method. Antimicrobial activity of mangosteen pericarp extract was recorded highest zone of inhibition against *Staphylococcus aureus* (11mm) while butterfly pea flower extract was recorded highest inhibition against *E. coli* (12mm). Mangosteen extract and butterfly pea extract were observed to have high-quality antioxidant activity. The IC₅₀ values of mangosteen extract was 51.53 µg/ml and butterfly extract was 92.42µg/ml evaluated. Based from the results methanol was proper solvent to extract the antioxidant antimicrobial compounds from *Clitoria ternatea* and mangosteen.

Keywords: Antioxidant, antimicrobial, DPPH, mangosteen, *Clitoria ternatea*

1. Introduction

On a daily basis consumption of fruits and vegetables have been epidemiologically helped with a less occurrence of mortality and morbidity via chronic degenerative diseases, such as hypertension, cancer, dyslipidemia, type 2 diabetes mellitus, cerebrovascular and cardiovascular diseases. This organization is powerfully evidenced by content of phytochemical compounds antimicrobial compounds, antioxidant and anti-inflammatory properties as phenol, Flavonoids, fibers, carotenoids and vitamins in these foods (Gregoris *et al.*, 2013; Pem and Jeewon, 2015; Oszmiański, *et al.*, 2015; Cavallo *et al.*, 2016) [4, 7, 19, 20]. Current scientific publications are some evidences and supported that phytochemicals presence in fruits played a valuable role in prevention and treatment of degenerative diseases.

Many compounds of dietary plants and medicinal plants have been known as possessing possible chemo-preventive properties accomplished of reversing inhibiting and retarding the multistage method. (Surh YJ *et al.*, 1998; Surh YJ, 2003; Li YY and Martin CP, 2010) [16, 25, 26]. The significant reward claimed for remedial use of medicinal plants in different ailments because of their protection besides being economical, effective and easy accessibility. (Atal CK and Kapoor BM, 1989; Siddiqui HH 1993; Singh A *et al.*, 2012) [2, 27, 28].

Medicinal agents derived from medicinal plants could be in the form of stem, fruit, leaves, roots and flowers which have payback as a medicine and also used as raw materials for manufacture of traditional medicine and modern medicine. (Agusta, 2000) [1] Medicinal plants are good another sources to find out remedies for accessible noncommunicable diseases over all world. (T. Efferth *et al.*, 2007) [32] In additional, several studies investigated that antioxidants rich foods play a critical role in prevention and management of oxidative stress related chronic diseases.

1.1 *Garcinia mangostana* L.

Mangosteen (*Garcinia mangostana* L.) belonging to Clusiaceae family and is a tropical queen of fruits, generally found in India and Southeast Asia. (Ji *et al.*, 2017) [13] Mangosteen parts such as seed, pericarp, leaves and bark, have been using for medicinal purposes for treatment of diarrhea and skin infections. Numerous compounds in the mangosteen pericarp have recently been identified such as polyphenolic compounds including xanthenes, anthocyanins, gartanin, gartinone E, 8-deoxygartanin and prenylated xanthenes (S. M. Petiwala *et al.*, 2014; Z. Xu *et al.*, 2014) [29, 37].

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Mangosteen pericarp produces xanthenes that are useful as antibacterial, antioxidant, antitumor, anti-allergy, antiviral and anti-inflammatory. (Chomnawang M T *et al.*, 2007; Pedrasa-Chaverri J *et al.*, 2008) [5, 21] Xanthone play role as antibacterial and gives the potential as a natural pesticide to organize pathogenic bacteria for plants. A number of antioxidants and bioactive compounds are found in mangosteen pericarp extract like oxygenated xanthenes, prenylated and xanthenes. (Zarena AS *et al.*, 2009; Widowati W *et al.*, 2014; Abuzaid AS *et al.*, 2016) [3, 35, 38] Xanthenes structure composed by tricyclic system (C6-C3-C6). The richest xanthenes in mangosteen rind are α - and γ -mangostin (Walker ED *et al.*, 2007) [36] and other xanthenes in mangosteen pericarp such as garcinones C, D, 8-deoxygartanin, β -mangostin, garcinones A, B and E, mangostinone, 9-hydroxycalabaxanthone and isomangostin. (Gutierrez-Orozco *et al.*, 2013) [8].



Fig 2: *Garcinia mangostana*

1.2 *Clitoria ternatea*

Clitoria ternatea belongs Fabaceae family. Generally called as butterfly-pea, blue-pea and cordofan-pea. Blue pea is a perennial herb found in India, Madagascar, China and Philippines. It is broadly originate in the low land tropics, humid, occurring naturally and in cultivated form (Devi *et al.* 2003, Gupta *et al.* 2010) [6, 9]. White and blue flower of butterfly pea are found in India, China, Madagascar and Philippines. It is popularly known as “Shankhpushpi” in India (Kulkarni *et al.* 1988) [14]. The plant extract has potential medicinal principles like inflammatory, antipyretic, anti-helminthic, anti-antibacterial, analgesic, anticonvulsant, anticancer, antidepressant, anxiolytic, sedative and hypoglycemic properties. (HariPriya *et al.*, 2010; Indumathi *et al.*, 2014; Kamtekar, S *et al.*, 2014) [10, 12, 15].

The richest source of unique anthocyanins along with secondary metabolites in *C. ternatea* makes the plant a good source of natural components that may enhance the nutritive values of consumer foodstuffs (Siti Azima *et al.*, 2017; Pasukamonset *et al.*, 2016, 2017, 2018) [22, 23, 24, 30].

2. Materials and Experiments

2.1 Plant Sample Collection

Mangosteen fruits were procured from fruit market Hyderabad and Butterfly pea flower was collected from botanical garden. Mangosteen pericarp and butterfly pea flowers were dried in shady place and kept in zipper pouch for further study in laboratory condition.



Fig 1: *Clitoria ternatea*

2.2 Microorganism culture

Four bacterial culture *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were maintained in nutrient agar slants and Luria Bertani agar slants at 4 °C. Two Gram's Positive and two Gram's Negative bacteria were used for antibacterial activity.

2.3 Extract preparation

The dried mangosteen pericarp and butterfly pea flowers were put separately into a grinder to produce powder and then extracted in methanol for 24 hours. Extracts were filtered and evaporated at 37 °C. Obtained residues were redissolved in respective solvents.

2.4 Phytochemical Screening

The samples were screened for alkaloids, flavonoids, carbohydrates, proteins, glycosides, phenols, tannins, quinines, amino acids, saponins, sterols, terpenoids confirm the presence of the secondary metabolites in the prepared extracts. (Treace GE 1989; Harborne JB 1973) [11, 33].

2.4.1 Test for alkaloids (Wagner's Test)

1ml of extract was treated with few drops of Wagner's reagent and formation of reddish brown precipitate or coloration was observed.

2.4.2 Test for Carbohydrates (Molisch's test)

1ml of extract and 2ml of conc. H₂SO₄ was added in the test tube. The mixture was allowed to stand for 2-3 mins. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

2.4.3 Test for Glycosides (Keller Kelliani's test)

1ml of extract and 0.5ml of glacial acetic acid was taken in a test tube then one drop of ferric chloride solution was added. Carefully added 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of Glycosides.

2.4.4 Test for Flavonoids (Alkaline reagent test)

2ml of extract was added few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, that becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

2.4.5 Test for Phenols (Ferric chloride test)

1 ml of extract was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

2.4.6 Test for tannins (Precipitate test)

2ml of extract was boiled with 1ml of 1% aqueous hydrochloric acid. Observed deposition of a red precipitate was taken the presence of tannins.

2.4.7 Test for Saponins (Foam test)

2ml of plant extract was mixed with 5ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for 5mins that confirms the presence of saponins.

2.4.8 Test for Sterols (Liebermann-Burchard test)

1ml of extract was treated with 1-2 drops of chloroform, acetic anhydride and conc. sulphuric acid and observed for the colour change in red or dark pink.

2.4.9 Test for Terpenoids (Salkowki's test)

1ml of chloroform was added 1ml of extract then added few drops of concentrated sulphuric acid and observed immediately formation of a reddish brown precipitate which indicated presence of terpenoids.

2.4.10 Test for Quinones

0.5 ml of extract was treated with concentrated HCl and observed for the formation of yellow precipitate or coloration.

2.5 Antimicrobial Activity

The antibacterial activity of mangosteen pericarp and butterfly pea flower were tested against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by using agar well diffusion method. (Sridhar TM *et al.*, 2011) [31] The bacterial inoculums were prepared and were adjusted to 0.5 McFarland turbidity standards (Laboratory Methods 2007) [17]. 100 µl of the suspension was streaked on Nutrient Agar to form lawn cultures. Four wells were punched on the surface of each culture plate with a sterile borer of 8 mm diameter. 30 µl of pericarp and flower extracts were poured into the wells. The plates were then placed at room temperature for an hour, followed by incubation for 24 hours at 37 °C. The degree of antibacterial activity against tested bacteria was observed by measuring the diameter of zone of inhibition. DMSO without the test ingredient was taken as a control. The tests were performed in triplicate.

2.6 DPPH radical scavenging activity

DPPH (1,1-diphenyl 2-picrylhydrazyl) free radical scavenging activity was measured according to Mäkyänen K (2013) [18]. The reaction mixture content follow as, the extract was added with 0.2 mM DPPH and incubated for 30 min at room temperature. The decrease in the solution absorbance was measured at 515 nm. The IC50 value was calculated from plots of log concentration of inhibitor concentration versus percentage inhibition curves. Ascorbic acid was used as a positive control for this study. The scavenging capabilities of DPPH radicals were calculated by the following equation.

$$\% \text{ Scavenging} = [(1 - (A_{\text{sample}}/A_{\text{control}}))] \times 100$$

3. Result and Discussion

3.1 Phytochemical Analysis

The results of the preliminary phytochemical analysis of mangosteen pericarp methanol and *Clitoria ternatea* flower methanolic extracts revealed the presence of different

bioactive compounds qualitatively such as proteins, carbohydrates, resins, alkaloids, tannins, steroids, phenols, flavonoids and glycoproteins were shown in table-1.

Table 1: Phytochemical Screening of *Garcinia mangostana* and *Clitoria ternatea*

Phytochemicals	Mangosteen pericarp Methanol Extract	Butterfly Pea Flower Methanol Extract
Phenols	+ve	+ve
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Carbohydrates	+ve	+ve
Glycosides	+ve	+ve
Tannins	+ve	+ve
Terpenoids	+ve	+ve
Saponins	+ve	-ve
Steroids	-ve	-ve
Quinones	-ve	-ve

3.2 Antimicrobial Activity

The antimicrobial activity of Mangosteen Pericarp Extract (Methanol) and *C. ternatea* flower extract (Methanol) were investigated for antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Mangosteen pericarp methanol extract shown highest zone of inhibition against (11mm) *Staphylococcus aureus* while *C. ternatea* methanol extract shown highest inhibition against (12mm) *E. coli*.

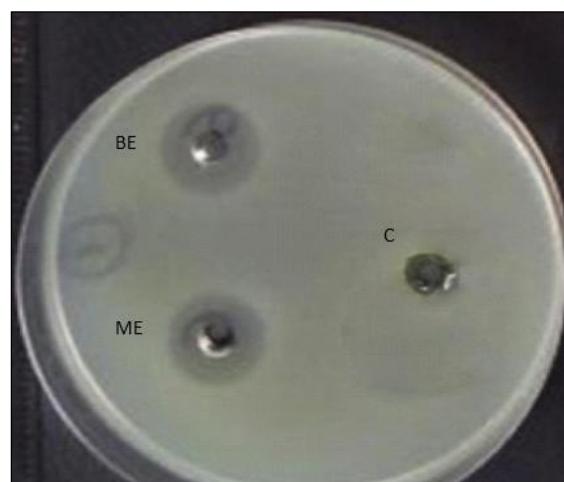


Fig 3: Antimicrobial Activity against *E. coli*

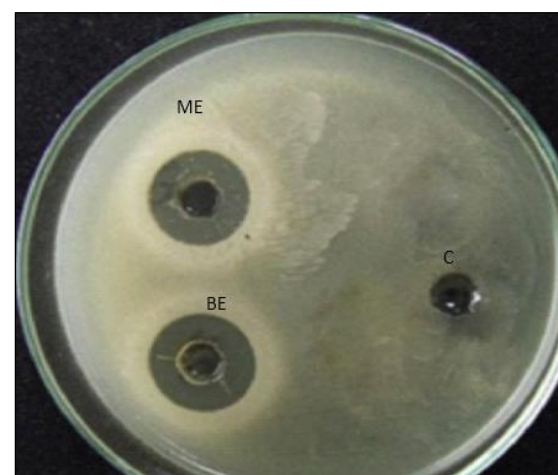


Fig 4: Antimicrobial Activity against *Staphylococcus aureus*

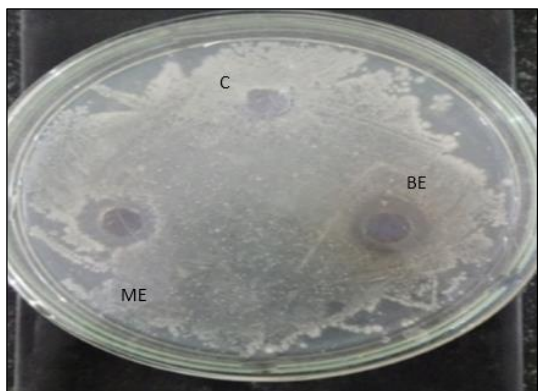


Fig 5: Antimicrobial Activity against *Pseudomonas aeruginosa*

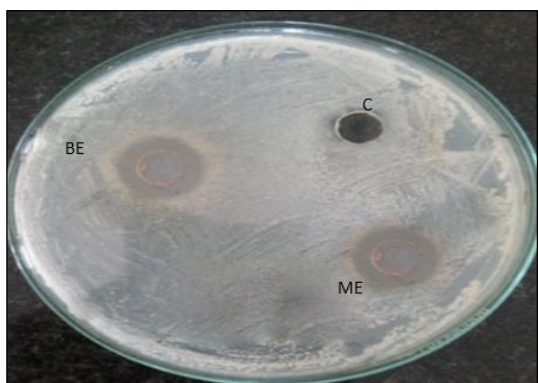


Fig 6: Antimicrobial Activity against *Bacillus subtilis*

Table 2: Antimicrobial Activity of Mangosteen and Butterfly Pea (Methanol Extract)

Strains	Mangosteen Pericarp Extract	<i>C. ternatea</i> Flower Extract
<i>Escherichia coli</i>	7 mm	12 mm
<i>Pseudomonas aeruginosa</i>	8 mm	11mm
<i>Bacillus subtilis</i>	10 mm	11.5 mm
<i>Staphylococcus aureus</i>	11 mm	10 mm

3.3 DPPH free scavenging activity

DPPH scavenging activity of mangosteen pericarp and butterfly pea flower were perented in Table 3. IC₅₀ and percentage of inhibition of extracts were shown in below Table. The antioxidants are understood to donate hydrogen from phenolic hydroxyl groups and break the free radical chain of oxidation forming a stable end product, which does not begin or propagate further oxidation. The DPPH radicals get stabilized by accepting the hydrogen donated by the hydroxyl groups present on the phenolic compounds. The data recorded show that the extracts are free radical scavengers and may act as primary antioxidants, which can react with free radicals by donating hydrogen.

Table 3: DPPH radical scavenging method (percentage inhibition) and IC₅₀ values

	Mangosteen Pericarp Extract		<i>C. ternatea</i> Flower Extract	
	IC ₅₀	% Inhibition	IC ₅₀	% Inhibition
DPPH	51.53	78	92.42	57

4. Conclusion

In this study, methanol extract of butterfly pea flower blue and mangosteen pericarp were investigated for the presence of phytochemicals, antimicrobial and antioxidant activity. The presence of different phytochemicals indicates that these

extracts compounds can be used as remedial drugs for, skin diseases insect bites, burning sensation, asthma, ascites, burning sensation, inflammation, leukoderma, leprosy, hemicranias and amentia. Further studies needs to explain the molecular mechanism of relations of plant based drugs with human body in diverse diseases.

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