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Phytochemical And Antibacterial Effect Of Dry Fruits Of *Garcinia Gummi-Gutta* (L.) Roxb

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Abstract: India is a rich repository of medicinal plants. Pathogenic microorganisms cause life threatening infections and its frequency may increase day by day and it increases morbidity and mortality in immune compromised patients. Among the different species of Garcinia, G. gummi-gutta is the most widely distributed Garcinia species in Kerala, south India. Garcinia gummi-gutta, popularly known as Malabar tamarind or kodampuli is a tropical fruit tree species of high potential and fruit is used as culinary spice, preservatives and also as a source of several nutraceutical products. The study was designed with an objective to evaluate the phytochemicals and antibacterial properties of *Garcinia gummigutta* fruit extracts. The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The preliminary phytochemical screening was carried out using standard methods. The dried fruits of *Garcinia gummi-gutta* were collected, dried, and extracted using hexane, ethyl acetate, chloroform, acetone and hydro alcohol solvents. The extracts of *Garcinia gummigutta* were tested positive for Carbohydrate, Terpenoids, Amino acids, Tannins, Cardiac glycosides, Flavonoids, Phlobatanins, Steroids, Phenolic compound and saponin. The prepared fruit rind extracts were examined to evaluate the antibacterial activity against both gram-positive (*Bacillus cereus, Staphylococcus aureus*) and negative bacteria (*Escherichia coli*) using agar disc diffusion assay. The zone of inhibition was measured.. The hydro alcohol extract is found to be most effective against the microorganism Hence, Garcinia gummigutta can be used to discover bioactive natural products that may serve as leads in the development of nutraceuticals or therapeutical agents to prevent several diseases.

Key words: *GGarcinia gummigutta*, Phytochemical, Chromatography, Microbes, Antibacterial activity.

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1. INTRODUCTION

Medicinal plants are considered as the "backbone" of traditional medicine, which means that more than 3.3 billion people in the developing countries utilize medicinal plants on a regular basis[1]. Medicinal plants or their parts are used as herbal preparations for the treatment of various ailments based on the experience passed from generation to generation. In order to promote the use of herbal medicine and the determination of their potential, the studies of medicinal plants should be more intensified[2] .The Genus *Garcinia* contains around 240 species, where 36 species are found in the two ecosystems of India, the Western Ghats and the Himalayan foothills[3]. *Garcinia gummi-gutta* (L.) Roxb. is a most common species found in Western Ghats. It belongs to the family Clusiaceae, a slow growing evergreen tree which grows in tropical areas of South Eastern and Eastern Asia, as well as in Western and Central Africa, although it is believed to have originated from the Indian subcontinent[4] . It is known by a variety of names including brindle berry, Malabar tamarind, and pot tamarind etc. Numerous scientific studies have indicated biological activity such as anti-obesity, hypercholesterolemia, antioxidant and anticancer activity[5] . The fruit of plant is a commercial product act as major market revenue, and is demanding for southern part of India.The phytochemical constituents include biflavonoid, xanthone and benzophenones and the principle acid in the fruit and rind is hydroxy citric acid. This acid has been found to suppress fatty acid synthesis, lipogenesis, food intake and to promote glycogenesis, while inducing weight loss. The sun-dried rind of the fruit is astringent, antiseptic and purgative. The efficacy of G. gummi-gutta on the lowering of fatty acid composition in mammals has already been reported.The plant *Garcinia gummi-gutta* (L.) Roxb has gained much importance as a promising source of ethno medicine. The people belonging to Kurichia, Kuruma, Kattunaika, Adiya and Paniya tribes of Wayanad use the plant in the treatment of many diseases[6] Hence, in the present study we tried to evaluate the phytochemical screening and *in- vitro* antibacterial activity of dry rinds of *Garcinia gummi-gutta* fruit extracts. The fruit rinds extracts were examined to evaluate the antimicrobial activity against two gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus)* and one gram-negative bacteria, *Escherichia coli*, using agar disc diffusion antimicrobial assay.

2. MATERIALS AND METHODS

2.1 Collection and processing of plant samples

The fruit rinds of *Garcinia gummi-gutta* were collected from Thattekad, Kerala.The fruit rinds of *Garcinia gummi-gutta* were washed with water, shade dried at room temperature and powdered coarsely. Exactly 15g of the coarse powder of fruit rinds were taken in 100 ml of various solvents such as ethyl acetate, hydro alcohol, chloroform, acetone and hexane. The extracts were refrigerated for 72 hours and filtered through Whatman filter paper No.1. Qualitative phytochemical tests and antibacterial study were conducted on these extracts[7]

2.2 Phytochemical screening procedure

The phytochemical components of the fruit extracts were screened by using standard procedures[8].

2.3 Tannins

To 1 ml of extract, add 2 ml of 0.1% Ferric chloride. Brownish green or blue black coloration shows the presence of tannins.

2.4 Saponins

Add 2 ml of distilled water to 1 ml of the extract, shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it confirms the presence of saponins.

2.5 Phenolic flavonoids

To 1 ml of extract add 5 ml of Folin ciocalteu reagent and 4ml of sodium carbonate. Appearance of blue color indicates the presence of phenol**.**

2.6 Flavonoids

Add 2 ml of 1% aluminium solution to 1 ml of the extract. Appearance of yellow color confirms the presence of flavonoids.

2.7 Carbohydrates

Add 5 ml of Benedict's reagent to 1 ml of extract and boil for 5 minutes. Bluish green color shows the presence of carbohydrates. b) Add few drops of Molisch's reagent and few drops of concentratedsulphuric acid to 1ml of extract which gives purple color.

2.8 Amino acids

Few drops of 0.2% ninhydrin was added to a 1ml of the extract and heated for 5 minutes. Formation of blue color shows the presence of amino acids.

2.9 Steroids

To 1 ml of the filtrate add 10ml of chloroform and 10 ml of sulphuric acid slowly by the sides of the test tube. Upper layer turns red and a sulphuric acid layer shows the yellow colour with green fluorescent is a positive indication.

2.10 Terpenoids

Take 1ml of filtrate, add 2ml of chloroform and carefully add a few drops of concentrated sulphuric acid. An interface with a reddish brown coloration is formed showing presence of terpenoids[9]

2.11 Cardiac glycosides

To 1 ml of extract, add 1ml of Ferric chloride reagent and few drops of concentrated sulphuric acid. Greenish blue color appears within a few minutes indicating the presence of cardiac glycosides.

2.12 Phlobatannins

Add a few drops of 1% aqueous hydrochloric acid to a 1 ml of the extract. A red precipitate is formed to confirm the presence of phlobatannins[10]

2.13 Chromatographic Analysis

The hydro alcohol, acetone, and chloroform extract was subjected to silica gel chromatographic separation using standard procedures.[11]The chromatographic column of length 50 cm and an internal diameter 3.5 cm was rinsed with acetone and allowed to dry. It was then clamped vertically with a retort stand, and a plug of cotton wool was inserted at the bottom of the column using a clean glass rod. The column was filled with hexane and the silica gel slurry. The slurry was poured into the column and tapped intermittently for uniform distribution of the gel and to avoid bubbles in the column. The column was allowed to drain for some minutes to stabilize it. A portion of three different crude extracts was subjected to chromatography in column silica gel. The column was eluted with solvent mixtures of increasing polarity. Fractions were collected and tested for antibacterial activity using both gram positive and gram negative bacteria.[12-13.]

2.14 Evaluation of antimicrobial activity

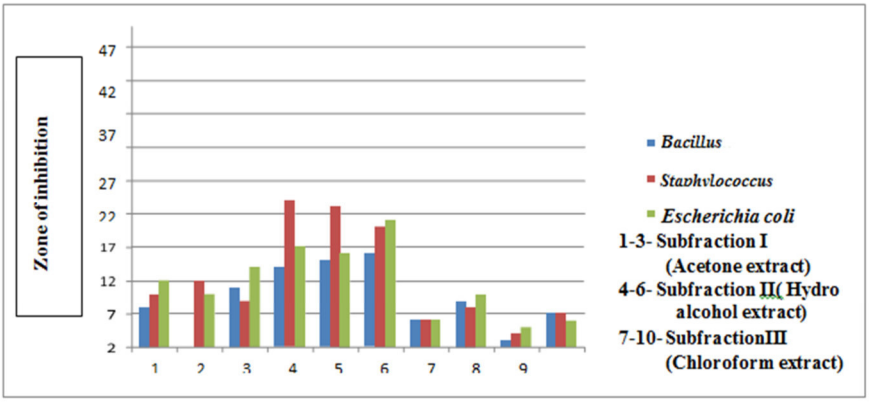
Antimicrobial activity of the fruit extracts was determined using a modified disc diffusion method[14-15]. 100 µl of the test bacteria were grown at 37 ^{0}C in 10 ml of fresh nutrient broth until they reached a count of approximately 10 ^{8} cells/ml (determined by direct microscopic counting). Himeda Agar (2%) was prepared and mixed with nutrient broth and added to Borosil petridishes. One hundred microliters of bacterial suspension were spread onto the appropriate agar coated plates (Borosil). The extracts were tested using 5 mm sterilised filter paper discs. Discs were impregnated with different fractions of elutions of three different fruit extracts, allowed to dry and placed onto inoculated plates. Plates were incubated at 37 ^{0}C for 24 hours. Diameters of the inhibition zones were measured in millimeters. Each antimicrobial assay was performed in at least triplicate and mean values were determined.

3. STATISTICAL ANALYSIS

The statistical analysis was done using SSPS Version 20.0. The bacterial inhibition rate was compared with respect to the control antibiotics. For this, Duncan method in SPSS was adopted for comparison. The data set with $p<0.05$ was ideally considered as significant.

4. RESULTS

The results of preliminary phytochemical constituents are tabulated in Table:1. In the present study, preliminary phytochemical analysis of *Garcinia gummi-gutta* fruits was done using different successive solvents like hexane, ethyl acetate, hydro alcohol, chloroform, and acetone. Carbohydrates are present in hydro alcohol and ethyl acetate extract. Terpenoids, Phenolic compounds and saponins are present in all the extract administered in the study. Amino acids, flavonoids and steroids are present in all the extract except in hexane extract. Tannin is present in ethyl acetate, acetone and chloroform extract and showed prominent color. Cardiac glycosides are present in all the extract except for chloroform extract. Phlobatanin was present in ethyl acetate and chloroform extract. Column chromatographic analysis of the three different extracts resulted in different fractions of elution with different quantities. Hydro alcohol and acetone extract showed three different fractions of elution, (B1, B2, B3) and A1,A2,A3 respectively. Chloroform extract gives four different fractions, (C1, C2, C3, C4) (Fig 2). Among the different fractions of elution of *G. gummi-gutta,* all elutes showed good to moderate antibacterial effect on all the strains that were tested, which were summarized in Table 2. For this study two gram positive bacteria (*B. subtilis, S. aureus*) and one gram negative bacteria (*E. coli)* were used. In acetone extract a third fraction (A3) showed a high zone of inhibition to *Bacillus* and *E.coli*. Fraction 2 (A2) showed a high zone of inhibition to *Staphylococcus.* The fraction three of hydro alcohol extract (B3) showed a high zone of inhibition to both *Bacillus* and *E.coli*. Fraction 1(B1) showed a high zone of inhibition to *Staphylococcus.* In Chloroform extract, fraction 2 (C2) showed a high zone of inhibition to *Bacillus, Staphylococcus* and *E.coli*. Antibacterial activity of Garcinia fruit was observed for hydroalcoholic extract with respect to the control antibiotics. B1 and B2 fraction from hydroalcoholic extract showed24±0.4^c and 23±0.6^{c*} against *Staphylococcus aureus.* Both fraction showed similar result to that of the standard (Gentamycin and Chloramphenicol). P value less than 0.05 was showed among fraction A1, A2, B1.B2,B3 and also among control (Vancomycin, G- Gentamycin, C- Chloramphenicol). Thus from statistical analysis, it can be concluded that the values showed significant variation between the groups (Table 2, Fig. 1).



Graph 1: Graphical representation of zone of inhibition of *Garcinia gummi gutta* on three bacterials

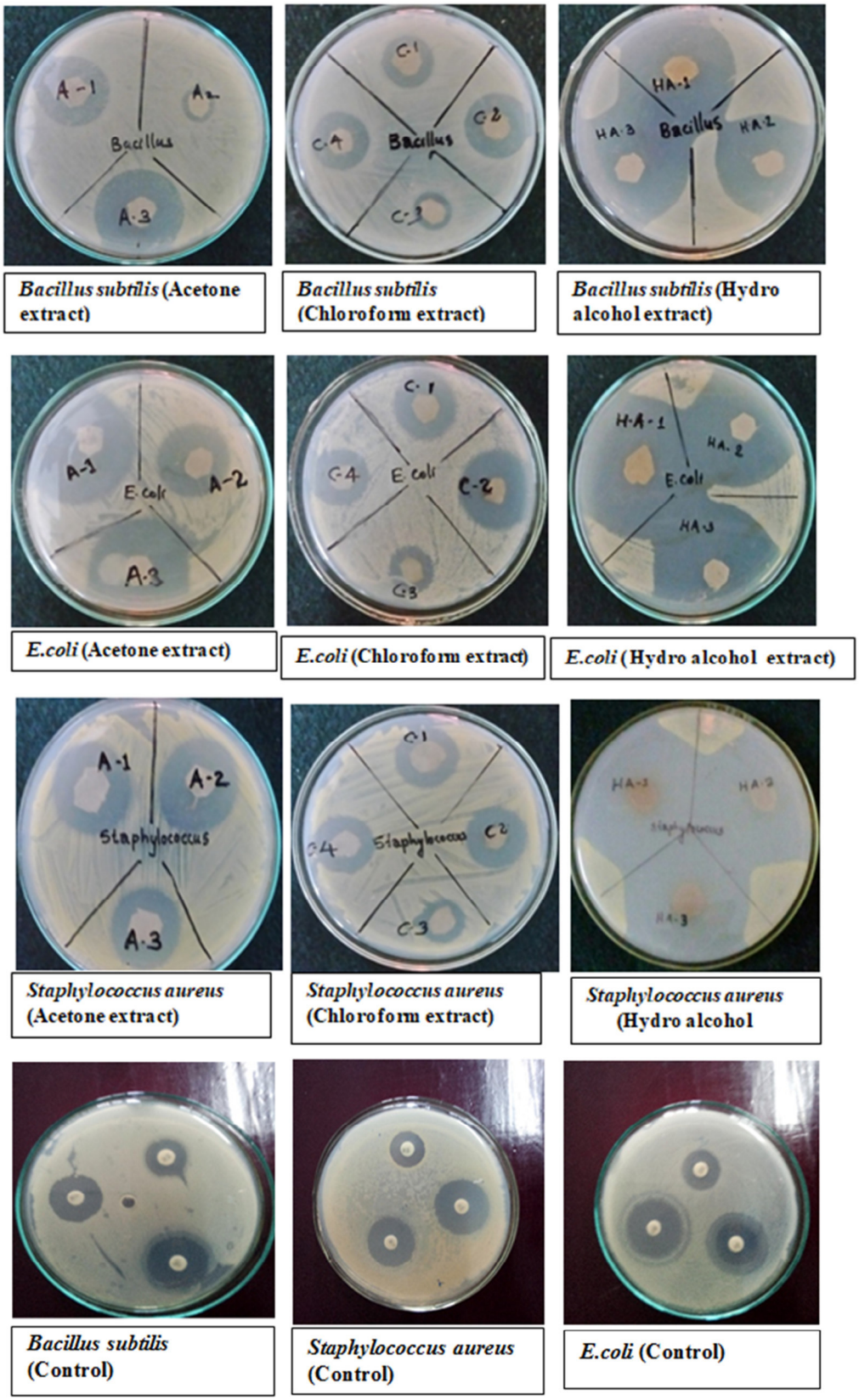


Fig 1. Zone of inhibition of dry rind of *Garcinia gummi gutta*

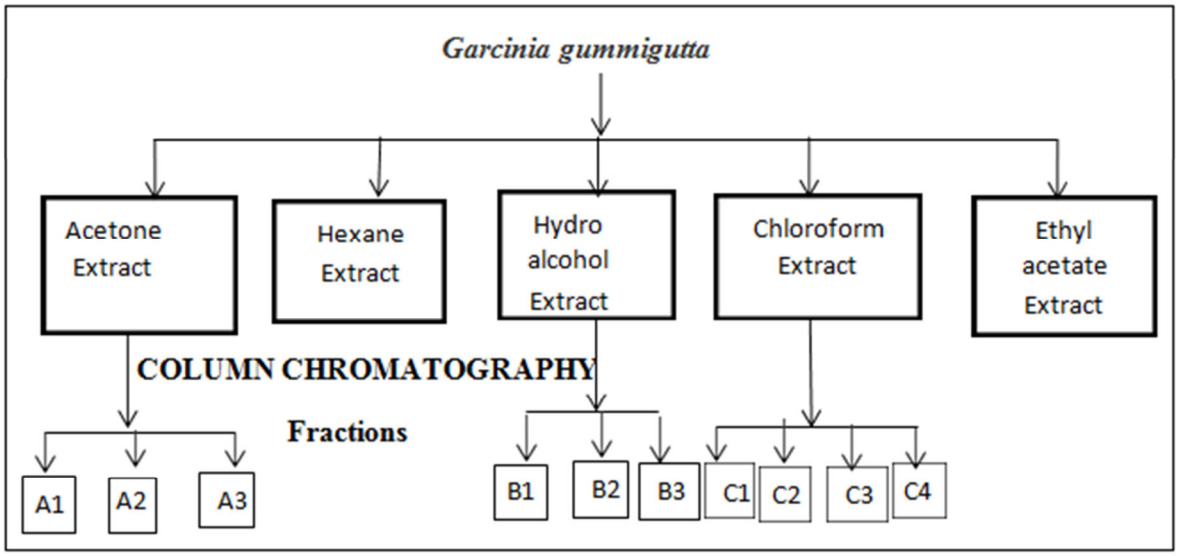


Fig 2. Schematic diagram showing isolation of compounds using column chromatography

Table 1:Phytochemical analysis of dry fruit of *Garcinia gummi-gutta*

SL NO:	NAME OF TEST	ETHYL ACETATE	HYDRO ALCOHOL	ACETONE	CHLOROFORM	HEXANE
1.	Carbohydrate	+	+	-	-	-
2.	Terpenoids	+	+	+	+	+
3.	Amino acids	+	+	+	+	-
4.	Tannins	+	-	+	+	-
5.	Cardiac glycosides	+	+	+	-	+
6.	Flavonoids	+	+	+	+	-
7.	Phlobatannins	+	-	-	+	-
8.	Steroids	+	+	+	+	-
9.	PhenolicCompound	+	+	+	+	+
10.	Saponins	+	+	+	+	+

+ = Present ; - = Absent. Based on the phyto chemical test colour was detected and denoted as (+) and the colour which was not detected was treated as (-)

Table 2 Antibacterial effect of dry fruit of *Garcinia gummi gutta*

S.no.	Organism	Zone of inhibition (mm)												
		Acetone Extract			Hydro Alcohol Extract			Control			Chloroform Extract			
		A1	A2	A3	B1	B2	B3	V	G	C	C1	C2	C3	C4
1	*BC*	$8\pm0.5^{a*}$	2 ± 0.4^{a}	11 ± 0.8^{bc}	14 ± 0.4^{a}	15 ± 0.2^{a}	$16\pm0.2^{ab*}$	16 ± 0.32^{ab}	28 ± 0.82^{b}	$21\pm0.25^{ac*}$	6 ± 0.6^{a}	9 ± 0.2^{b}	3 ± 0.2^{a}	7 ± 0.3^{b}
2	*SA*	10 ± 0.6^{b}	12 ± 0.6^{a}	9 ± 0.5^{b}	$24\pm0.4^{c*}$	$23\pm0.6^{c*}$	$20\pm0.4^{e*}$	$17\pm0.34^{ab*}$	$25\pm0.2^{a*}$	$24\pm0.42^{ab*}$	6 ± 0.21^{a}	8 ± 0.2^{ab}	4 ± 0.3^{b}	7 ± 0.2^{b}
3	EC	12 ± 0.5^{a}	10 ± 0.7^{b}	14 ± 0.3^{a}	$17\pm0.5^{a*b}$	16 ± 0.4^{b}	21 ± 0.5^{c}	18 ± 0.41^{c}	28 ± 0.53^{b}	34 ± 0.23^{d}	6 ± 0.56^{a}	10 ± 0.3^{c}	5 ± 0.5^{c}	6 ± 0.4^{a}

Mean ± SD and p<0.05.

BC- Bacillus cereus, SA- Staphylococcus aureus,EC- Escherichia coli, V- Vancomycin (10 mg), G-Gentamycin(10 mg), C- Chloramphenicol(30mg). Each value is expressed as mean± std, n=3. a-d superscript denote, different letters in the same column represent significant statistical difference p<0.05 by Duncan multiple range test in SPSS version 20.0. Mean values followed by different superscript in the columns are significantly different.*Represent p<0.05 in the givn data.

5. DISCUSSION

The World Health Organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicines mostly plant -based drugs[16]. The results of preliminary phytochemical constituents are tabulated in Table:1. In the present study, preliminary phytochemical analysis of *Garcinia gummi-gutta* fruits was done using different successive solvents from polar to non-polar solutions. The and nonpolar solvent ethyl acetate showed detection of colour with secondary metabolite reagent. Similarly. non-polar solvents showed detection of secondary metabolites (Terpenoids, cardiac glycosides, saponins and phenolics), except primary metabolites (Carbohydrates).The study was similar to the finding o, that secondary metabolites like phenolics, terpenoids are found in ethylacetate and hydroalcoholic extract [17] Flavonoids are present in hydro alcohol, ethyl acetate, chloroform and acetone extract of

low molecular weight polyphenolic compounds which include flavones, flavonoids, isoflavones, flavonols, flavan- 3-ols and anthocyanins. It was widely distributed in plants fulfilling many functions[18] . Flavonoids are generally non- nutritive agents. They possess remarkable antioxidant activities and inhibit enzyme activities like lipoxygenase, cyclooxygenase and prostaglandin synthase. Flavonoid compounds have proved of greater general interest to the plant taxonomist, both in respect of general angiosperm taxonomy and for detailed studies of gene flow at the specific and intraspecific levels[19]. Extensive biological roles of flavonoids have been reported which include antiviral, anti-hepatotoxic, therapeutic, antibacterial, and other roles in nature. The presence of flavonoids enhances the physiological survival of plants by shielding them from parasitic diseases and UV radiations. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes[20] . The detection of flavonoids in both, polar and non-polar solvents clearly support that, these compounds are responsible for the biological activity especially antimicrobial function. Phenols are found in the natural world, especially in the plant kingdom. The antioxidant activity of phenol is mainly due to their redox properties, hydrogen donor and singlet oxygen quenchers. Some phenols are proved to have hypotensive effects and antioxidant properties. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[21]. Naturally antioxidant in plants is in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc[22]. Biological activities of phenolic compounds involve free radical scavenging in cells[23]. Phenolics are present in all the extract and it plays important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Importantly, phenolic phytoalexins, secreted by wounded or otherwise perturbed plants, repel or kill many microorganisms, and some pathogens can counteract or nullify these defenses or even subvert them to their own advantage. In the present study, phenolic compounds were detected for ethyl acetate, hydro alcohol, acetone, chloroform, and hexane extract of fruit Garcinia gummi-gutta. The study showed positive results for terpenoids in ethyl acetate, hydro alcohol, acetone, chloroform and hexane extract for fruit. Terpenoids (isoprenoids) represent the largest and most diverse class of chemicals among the myriad compounds produced by plants[24] Plants employ terpenoid metabolites for a variety of basic functions in growth and development but use the majority of terpenoids for more specialized chemical interactions and protection in the abiotic and biotic environment. Tannins are another group of phenolic compounds and are present in ethyl acetate, chloroform and acetone extracts. Tannins play an effective role in protecting the kidneys. Tannins are a group of natural products widely distributed in plants. They are currently investigated for human medicinal use[25] to help reduce the risk of coronary heart diseases[26]. They are divided into two basic groups such as hydrolysable and condensed type. Hydrolysable tannins are normally recommended for treatment of inflammation, ulceration and tropical application for skin diseases.Tannins have shown potential antiviral, antibacterial and antiparasitic effects. Thus, detection of tannin in preliminary phytochemical study clearly indicated that this fruit is pharmaceutically important. Cardiac glycosides are a unique group of secondary metabolites that are considered one of the most useful drugs in therapeutics, and are present in all the extracts except chloroform. Saponins present in plants have been suggested as possible anti- carcinogens. However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied[27] . It shows beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Detection of saponins in selected plant may serve as anti- feedants, and to protect the plant against microbes and fungi. Benedict's test shows the presence of carbohydrate in the hydro alcohol and ethyl acetate extracts. In hydro alcohol, ethyl acetate and chloroform extract of fruit *Garcinia gummi-gutta* showed the presence of amino acids. Phlobatannin is another group of secondary metabolites, have diuretic properties and from the study both Ethyl acetate and chloroform extract shows the presence of phlobatannins. Similarly, steroids were also detectedin hydro alcohol, ethyl acetate, acetone extracts and chloroform extract of fruit. The present study reveals that all the extracts have significant amounts of phytochemical properties.Column chromatographic analysis of the three different extracts resulted in different fractions of elution with different quantities. Hydro alcohol and acetone extract showed three different fractions of elution and chloroform extract gives four different fractions (Fig 2). The different fractions of elution of *G. gummi-gutta* showed good to moderate antibacterial effect on all the strains that were tested, which were summarized in Table 2. For this study two gram positive bacteria (*B. subtilis, S. aureus*) and one gram negative bacteria (*E. coli)* were used. The hydro alcohol extract was superior in antibiosis, inhibiting the growth of test bacteria with wider zones of growth inhibition. Compared to three bacteria, the second fraction eluted from Hydroalcohol extract showed very good antibacterial activity on *Staphylococcus aureus* and the zone of inhibition. This result was similar to control (Gentamicin, vancomycin and chloramphenicol) used in the bacterial study (Table 2). It is evident from the results of this study that the plant *Garcinia gummi gutta* has immense potential as a medicinal plant, rich in bioactive compounds, and having significant levels of antibacterial activity[28]Separation of fractions from *Garcinia* and testing its antibacterial activity is done for first and no much reports are observed. The plant has gained much importance as a promising source of ethnomedicine. The study should be extended to more number of pathogenic bacteria. Further attempts should be made to selectively isolate and identify the active compounds of the extract and determine the rate of toxicity, their mode of action and dose dependent activity against various strains of bacteria. In future it can be used as a good bactericidal plant drug.

6. CONCLUSION

The present study has profiled biologically active phytochemicals of *Garcinia gummi gutta* in the ethyl acetate, hydro alcohol, acetone, chloroform and hexane extracts. Terpenoids, phenolic compounds, and saponins are present in all the extracts. The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. *Garcinia gummi-gutta* showed a significant antibacterial activity. Acetone, hydroalcohol and chloroform extract of *G. gummi gutta* showed antibacterial activity against *Bacillus*, *E.coli*, and *Staphylococcus*. The Hydro alcohol extract was superior in antibiosis, inhibiting the growth of test bacteria with wider zones of growth inhibition. Further research is needed for the isolation and identification of active principles present in

the extracts which could possibly be exploited for pharmaceutical use.

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8. AUTHORS CONTRIBUTION STATEMENT

Juliya V Devasia and Pinkie Cherian conceived of the presented idea. Juliya developed the theory and performed the computations. Pinkie verified the analytical methods. Pinkie encouraged Juliya to investigate and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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