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Anti microbial and anti-oxidant properties of the isolated compounds from the methanolic extract from the leaves of *Tectona grandis*

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ABSTRACT

The compounds Gallic acid (GA), rutin(R), quercitin (Q), ellagic acid (EA) and sitosterol(S) were isolated from the methanolic extract of the leaves of *Tectona grandis*. These compounds were subjected to antimicrobial and antioxidant activity. The zone of inhibition of isolated compounds was evaluated by cup plate method against bacteria i.e. *Staphylococcus aureus*, Bacillus *subtilis, Eschericia coli, Klebsiella pneumoniae* and fungi *Candida albicans*. The anti oxidant activity of the extract and the isolated compounds were evaluated by using 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH). Rutin has shown significant anti microbial activity against both the gram positive and gram negative bacteria when compared to the other compounds. The results of the anti oxidant activity revealed that quercitin showed good activity followed by rutin gallic acid, ellagic acid and sitosterol. The difference in both these activities of the isolated compounds was attributed to the number and position of the phenolic OH groups.

KEY WORDS

Tectona grandis, antimicrobial, antioxidant, rutin

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INTRODUCTION

Medicinal plants used in traditional Ayurveda and Unani medicine are effective in treating various ailments caused by bacterial and oxidative stress. *Tectona grandis*; belonging to the family Verbinaceae has been used for various disorders such as in the treatment of urinary discharge, in the treatment of the common cold and headache, as a laxative and sedative, in bronchitis, as diuretic, anti diabetic, in scabies, in wound healing, analgesic and anti inflammatory [1-5]. It is known fact that there is an increase in the infection rates due to the development of resistance by the microorganisms hence there is need to develop new antimicrobial drugs. Free radicals are responsible for various diseases like cancer; heart diseases etc. Number of plants have been reported to posses anti oxidant and antimicrobial activities [6,7,8].It has been reported that phenolic compounds show anti microbial activity against a wide range of microorganisms . They also posses significant antioxidant activity by virtue of the presence of the free phenolic groups [9]. We have already reported the wound healing activity of the isolated compounds [10]. The purpose of the present study was to investigate the antioxidant and antimicrobial properties of the isolated constituents of the plants i.e. two flavonoids (rutin, quercitin), two phenolic acids (gallic acid and ellagic acid) and a sterol (sitosterol), which posses phenolic OH in varying numbers and at different position. This paper reports the results of the studies which may be of significantly important in future investigations towards the development of potent and safe antioxidants and antimicrobials.

MATERIALS AND METHOD

Plant material

The frontal leaves of *Tectona grandis* were collected from the rural areas of Bangalore. Identified and authenticated by the Regional Research Institute, Bangalore where the specimen voucher (RRCBI Acc no 12474) has been deposited. The material was shade dried, pulverized and preserved in air tight containers.

Chemicals and reagents

Nutrient broth (NB), Nutrient agar (NA), Sabouraud Dextrose broth (SDB), Sabouraud Dextrose Agar (SDA), Peptone water and antibiotics flucanazole and streptomycin were procured from Hi-media laboratories, Mumbai, India. DMSO and other chemicals used for extraction and isolation of the compounds were procured from E.Merck Ltd.

Test organisms

The test organisms *Staphylococcus aureus* (NCIM 2079),*Bacillus subtilis* (NCIM 2063) *Eschericia coli* (NCIM 2065), *Klebsiella pneumoniae* (NCIM 2957), *Candida albicans* (NCIM 2325) were obtained from U-Win sciences, Bangalore.

Preparation of the extracts

The methanolic extract of dried powder (1 kg) of the leaves was prepared by using Soxhlet apparatus which was then concentrated and dried to give dark brown mass.

Phytochemical analysis and isolation

The extract was then subjected to preliminary phytochemical analysis using standard procedures [11].The compounds were isolated from the different fractions of methanolic extract by eluting the column with different mobile phases with gradual increase in polarity, starting from petroleum ether, chloroform, ethyl acetate and methanol.

Anti microbial studies of isolated compounds by cup plate method [12]

Cultures were sub cultured in NA/SDA plates and further stored in slants as stock cultures. For the evaluation, the stock culture was prepared by inoculating each culture from slants to flask in sterile NB/SDB and incubated at 37^0C/28^0C for 24/48 h. The stock culture was serially diluted by ten fold with sterile peptone water and 0.1ml from each dilution was spread over NA/SDA plates and incubated at 37^0C/28^0C for 24/48 h. The numbers of colony forming units (CFU) were counted from plates of each dilution and there by the total CFU were calculated in the stock culture. For antimicrobial screening the stock cultures of $1x10^5$ CFU per ml were used.

Determination of microbial growth inhibitory properties

Initial microbial growth inhibitory properties of test substances were determined by cup plate method .The drugs were dissolved in H_2O / DMSO and tested at concentration of 200 and 100 µg/ml against all the microorganisms.

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Sterile NA/SDA plates were prepared and 0.1 ml of the inoculum from the standardized culture of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10mm and 100µl of the test substance, standard antibiotic and the solvent control were added in each well separately. Amoxycillin and Flucanazole were used as standards and tested against bacteria and fungi, respectively. The plates were placed at 4^0C for 1 h to allow the diffusion of test solution into the medium and plates were incubated at a temperature optimal for 24 hours for bacteria at 37°C and 48 hours for fungi at 27°C. The zone of inhibition of microbial growth around the well was measured in mm.

Anti oxidant activity of the isolated compounds [13]

The anti oxidant activity of the extract and the isolated compounds were evaluated by using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH).The stock solution of the extract and the isolated compounds were prepared (10 mg/ml) in methanol. The working solutions (10, 20, 40, 80,100,120,140,180,200 and 250 mcg/ml) of the extracts and the isolated compounds were prepared from the stock solution using suitable dilutions. The anti oxidant activity of the plant extract and the isolated compounds were determined based on the radical scavenging effect of the stable 1,1-Diphenyl-2-picryl-hydrszyl (DPPH).The diluted working solutions of the samples were prepared in methanol. DPPH was prepared as 0.002% solution in methanol and mixed with 1ml of both the standard and the samples. The prepared solutions were kept in the dark for ½ hour and the absorbance was measured at 517nm.A mixture of 2ml methanol with 2ml of DPPH was used as a blank. The % absorbance was calculated using the formula

$$\% \text{ Absorbance} = \frac{A - B}{A} \times 100$$

where A is the absorbance of the blank and B is the absorbance of the sample.

RESULTS

Anti microbial effect of compounds by cup plate method

Gallic acid exhibited activity against *Bacillus subtilis*, *Eschericia coli*, and *Klebsiella pneumoniae* at 200 mcg/ml. Quercitin showed activity only against *Staphylococcus aureus and Klebsiella pneumoniae* at 200 mcg/ml. Rutin showed significant activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Eschericia coli*, *Klebsiella pneumoniae* bacteria at 200 and 100 mcg/ml, while ellagic acid showed activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae and Eschericia coli*, sitosterol showed activity at against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* at 200 mcg/ml.

Table 1: Anti microbial effect of test drugs at 200 mcg/ml and 100 mcg/ml by cup plate method.

Sl. no	Sample	Zone of inhibition in mm									
		Staphylococcus aureus		Bacillus subtilis		E. coli		Klebsiella pneumoniae		Candida albicans	
		200	100	200	100	200	100	200	100	200	100
1	Gallic acid	NI	NI	12	NI	12	NI	13	NI	NI	NI
2	Guercitin	11	NI	NI	NI	NI	NI	15	13	NI	NI
3	Rutin	17	13	15	12	14	11	14	NI	NI	NI
4	Ellagic acid	16	NI	15	NI	17	NI	17	12	NI	NI
5	Sitosterol	12	NI	13	NI	NI	NI	12	09	NI	NI
Standard drugs	Streptomycin (100 mcg/ml)	31	15	27	15	29	16	28	18	-	-
	Flucanozole (25mcg/ml)	0.96	278.5							18	14

NI: No Inhibition.

Table 2: Anti oxidant activity of the isolated compounds (absorbance and % inhibition).

Mcg/ml	Extract		Gallic acid		Rutin		Quercitin		Ellagic acid		Sitosterol	
	AB	% inh	AB	% inh	AB	% inh	AB	% inh	AB	% inh	AB	% inh
10	0.080	75.60	0.049	85.06	0.032	90.24	0.027	91.76	0.051	84.45	0.162	50.60
20	0.062	81.09	0.043	86.89	0.029	91.11	0.025	92.37	0.0 49	84.06	0.159	51.52
40	0.056	82.92	0.039	88.10	0.028	91.46	0.023	92.98	0.0 45	86.28	0.1 48	54.87
80	0.049	85.06	0.038	88.41	0.027	91.17	0.021	93.35	0.0 43	86.89	0.1 45	55.79
100	0.043	86.89	0.035	89.32	0.025	92.37	0.020	93.90	0.0 40	87.80	0.1 42	56.70
120	0.035	89.92	0.030	90.85	0.023	92.29	0.019	94.20	0.0 39	88.41	0.1 40	57.31
140	0.031	90.54	0.027	91.17	0.021	93.35	0.018	94.51	0.0 36	89.02	0.1 39	57.62
180	0.026	92.07	0.027	91.17	0.019	94.20	0.017	94.81	0.0 35	89.32	0.1 31	60.06
200	0.024	92.68	0.026	92.07	0.018	94.45	0.017	94.81	0.0 34	89.63	0.1 28	60.97
250	0.021	93.59	0.025	92.37	0.018	94.45	0.017	94.81	0.0 31	90.54	0.1 25	61.89

Table 3: IC_{50} of the methanolic extract and the isolated compounds.

Tested compounds	IC_{50}
Extract	63.00
Gallic acid	62.69
Rutin	61.01
Quercitin	60.38
Ellagic acid	64.08
Sitosterol	96.76

Anti oxidant activity of the isolated compounds

The results of the anti oxidant activity revealed that quercitin showed best activity followed by rutin , gallic acid, ellagic acid and sitosterol

DISCUSSION

Anti microbials of plant origin are effective in the treatment of several infections. The action of compounds containing phenolic hydroxyl groups may be related to inhibition of hydrolytic enzyme or other interactions to inactivate microbial adhesions, on specific transport of carbohydrates etc. The presence of the hydroxyl group and a system of delocalized electron are important for the antimicrobial activity [14]. Phenolic compounds exhibit a wide range of anti-allergenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects. Rutin has shown significant anti microbial activity against both the gram positive and gram negative bacteria when compared to the other compounds. Free radical scavenging is one of important routes by which damage to cells can be prevented. Increased production of reactive oxygen species (ROS) results in oxidative stress and in cytoxicity. The effect of antioxidants molecules on DPPH is due to their hydrogen donating ability. DPPH free radical method is an easy, rapid and sensitive method to evaluate the anti oxidant properties of extracts and isolated compounds. The anti oxidant study shows that the extract and the compounds have significant anti oxidant activity. Quercitin showed best antioxidant activity when compared to the other compounds, followed by rutin, gallic acid, ellagic acid and sitosterol. The radical scavenging activity of the compounds is related to the position and the number of free hydroxyl groups .The strong anti oxidant activity of the polyphenols is due to their action as scavengers of ROS, peroxide decomposers, quenching of singlet oxygen, electron donor and inhibitors of lipoxygenase. The isolated compounds all contain hydroxyl groups in varying numbers at different positions which are responsible for the difference in their anti oxidant activity. Flavonoid rutin differs from quercitin in the presence of a sugar rutinose at position 3, so it is possible that the sugar moiety could be contributing to the pharmacokinetic factor. It has been reported that although glycosides are usually weaker antioxidants than aglycones, their bioavailability is increased due to the increase in solubility by the presence of a sugar moiety. The type of sugar moiety also plays an important role in the activity i.e. glucose, rhamnose, or rutinose. For example, instead of rutinose, if rhamnose moiety is attached to quercitin it significantly reduces scavenging of radicals. Both quercitin and rutin are highly effective chelators of transition metals indicating that their is little difference between aglycones and glycosides in the ability to complex metal [15, 16].

CONCLUSION

The isolated compounds were subjected to anti microbial and anti oxidant activities using standard methods. The difference in the activities was attributed to the number and position of the phenolic groups present. This result of this study indicates and confirms that the structure plays an important role in the activity that is exhibited by the compounds.

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