Available Online at http://www.resealert.com

International Journal of Current Advanced Research
Vol. 1, Issue, 1, pp.5 - 9, September, 2012

International Journal of Current Advanced Research

RESEARCH ARTICLE

Evaluation of Antibacterial activity and Phytochemical Analysis of the leaf extracts of Alpinia*galanga* (L.) Willd

Deepa, S[1] and Prabakaran, G[2]

[1]Research Development Centre, Bharathiyar University, Coimbatore, 641046, Tamilnadu
[2]PG& Research Department of Botany, Government Arts College, Dharmapuri-636705.Tamilnadu

*Corresponding author- G.Prabakaran, Email- gpbiotek@gmail.com

ARTICLE INFO

Article History:

Received 10th August, 2012
Received in revised form 25th, August, 2012
Accepted 15th September, 2012
Published online 27th September, 2012

Key words:

Alpiniagalanga, galangal extract, disc diffusion, antibacterial activity, phenolic compounds

ABSTRACT

The antibacterial activity of *Alpiniagalanga*(L.)Willd. leaf extracts prepared from methanol, acetone, chloroform, ethanol, petroleum ether extracts of*Alpiniagalanga* were checked against pathogens isolated from wound isolates like Burn, Accident, Skin, Abscess, trauma etc viz. *Bacillus subtilis ,Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, , Staphylococcus aureus and Streptococcus*sps, *Proteus sps, Staphlococcusaureus*, using Agar well diffusion method. Acetone extracts have shown excellent anti bacterial activity towards all the pathogens with plant extract ranging from 5-25mg/ml and size of zone (mm) ranged 8-26 mm (12.5 mg) concentration and 8-35 mm (25 mg), respectively. The ethanolic extract contained the highest concentrations of total phenolic compounds (92.40 ± 0.83(mg TAE/g) and flavonoids (13.78 mg TAE/g) and Tannins 48.80 ± 0.83(mg TAE/g). So, *Alpiniagalanga* can be quite resourceful for the development of new generation drugs.

INTRODUCTION

Alpiniagalanga, (also *Languasgalanga*), is one of the most outstanding plant species[1, 7] .It is a member of Zingiberaceae family, is an herb used in cooking, especially in Indonesian cuisinewith the common name as greater galangal. Galangal has characteristic fragrance as well as pungency; hence, itsrhizomes are widely used as a condiment for foods in Thailand. Galangal is also used as a medicine for curing stomachache in China and Thailand (Yang &Eilerman, 1999). The leaves are broadly lanceolate, 30-60cm long and 10-15cm broad. The flowers are arranged in erect, terminal panicles composed of numerous spreading dichotomous branches each with two to six, pale greenish-white faintly fragrant flowers. Fruits 1.25 cm long, oblong, constricted in the middle or even pear shaped, three sided and deep orange red in colour. Seeds are ash coloured, three angled, finely striated towards the hilum. Both the seeds and rhizomes have pungent aroma (Thakur *et al*, 1989). The rhizome is used against rheumatism, bad breath and ulcers,whooping colds in children,throat infections and fever.1-acetoxychavicol acetate ,a component of A.galanga was foundto have a very good antimicrobial activity (J.Shivkanya, I.Nitin et al, 1990).The rhizome is an abortifacient, carminative, antituberculosis and also has stimulant properties. Ground rhizome is used in the treatment of skin infections such as eczema, ringworm, etc. A mixture of galangal and lime juice is used as a tonic in parts of Southeast Asia. The essential oil of A.galanga rhizome has been found to have inhibitory activity against certain dermatophytes,filamentous fungi and yeast (J.Oonmetta-areeet al ,2006).It tones up the tissues and is sometimes prescribed in fever. Homoeopaths use it as a stimulant. It has some reputation as a remedy for perineal relaxation with haemorrhoids and for a lax and pendulous abdomen. It is used as a snuff to treat cold and flu symptoms. Galangal Root has also been used as a digestive aid, especially in combating dyspepsia and flatulence.. It is used against nausea, flatulence, dyspepsia, rheumatism, catarrh and enteritis. It also possesses tonic and antibacterial qualities and is used for these properties in veterinary and homeopathic medicine. Suganya and Sombat have reported that the higher potential in antioxidant and antimicrobial activities of *A. galanga*oil was supposed to be due to the composition of certain constituents viz. 1, 8-cineole, 4-allylphenyl acetate and β-bisabolene within the essential oilV. Vudhakul et al, 2007). The pharmacognosy and toxicology of anti-carcinogenic natural products from galanga root oil has been studied by Zheng*et al* (1993).

The essential oil of *A. galanga*leaves is rich in 1, 8-cineole (28.3%), camphor (15.6%), pinene (5.0%), (*E*)-methyl cinnamate (4.6%), bornyl acetate (4.3%) and guaiol (3.5%).These are the general data given on the rhizome, root parts. Present study was taken up to evaluate the antimicrobial

activity of Alpinia galangal leaves and to establish its phytochemical constituents.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Alpiniagalanga*were collected and taxonomical identification of the plant was confirmed by Dept of Biotechnology G.K.V.K, Bangalore. The collected plants were washed with running water, dried, homogenised to a fine powder and stored in air tight bottles at 4^0 C.

Preparation of Plant Extract

The fresh plant leaf was harvested, rinsed with tap water and air dried under shade for 14 days and reduced to coarse powder and grinded to fine powder. The powder was stored in an airtight bottle until needed for use.

Preparation of Solvent Extract

Sample (50 gm) of the shade-dried powder of *Alpiniagalanga* was extracted in a Soxlet extractor successively with 200 ml Petroleum ether, Chloroform, and acetone until colourless extract was obtained on the top of the extractor. Each of the solvent extract was concentrated separately under reduced pressure. After complete solvent evaporation, each of these solvent extracts was weighed and subjected to antibacterial activity. Extracts were maintained at a temperature between 2 - 8°C for further studies (Mohana *et al.*, 2008).

Collection of wound pus samples

A totally 35 pus swabs were obtained from wound samples like burns, Accident wounds, trauma, Abscess, Skin infection. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. All samples were collected from in around Namakkal area hospitals and properly labelled indicating the source and age of patients. The samples were transported to the laboratory soon after being obtained. In the laboratory, the specimens were registered and swabs were cultured on nutrient broth and incubated at 37ºC for 24 hrs. Bacterial isolates were identified based on Colonial Appearance, Pigmentation and morphological characteristics using gram and Ziehl-Neelson staining procedures and Biochemical test were done using IMVIC tests**(** Cowan (1985), Fawole and Oso's (1988) and Cheesbrough (2004).

Antibacterial activity of plant extract against wound infecting bacteria

Antibacterial activities of crude plant leaf extracts were examined by the well diffusion method. Each plant extract was dissolved in respective solvents such as Acetone, Chloroform, Ethanol, Hexane, and Petroleum ether tested they were evaluated for each bacterium. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zone.

Experimental procedure: Well diffusion method (zone of inhibition)

The plant materials extracts were tested for antimicrobial activity by the well diffusion method (Chung et al., 1990). This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish to an extract such that growth of the added microorganism is prevented entirely in circular area or zone around the cavity containing the extracts (Cote and Ghemal 1994). Using micropipette 0.5 ml of each of the leaf broth containing 10-5-10-6 cfu/ml test organisms were incubated on the four plates of solidified agar and was spread uniformly with a glass spreader. Then four well were cut out in the agar layer of each plate with an aluminium bore of 5 mm diameter to contain 0.5 ml extract, standard drug. All the work was carried out in freeze for one day. After addition to allow diffusion of the solution in to the medium and then incubated for 37º C for 24 hours for antibacterial activity. After the incubation period the mean diameter of the zone of inhibition in mm obtained around the well was measured. Ampicillin was used as standard drug for antibacterial activity.

Phytochemical Screening of Alpinia galangal leaf extract

The leaf extract of *Alpiniagalanga,* were analyzed for the presence of Saponins, Phenolic compounds, Alkaloids, Flavonoids, Glycosides , Starch, Phenols and Tannins, Carbohydrates, proteins and Amino Acids,Steroids& Sterols. (Horbone (1998) and Kokate et al.,(1995) Table-2

Determination of total phenolics and tannins

The total phenolic content was determined according to Siddhuraju and Becker (2003). 10 µl aliquots of the etanolic leaf extracts (10mg/2ml) were taken in the test tubes and the volume was made up to 1 ml with distilled water. 0.5 ml of Folin-Ciocalteu phenol reagent (1:1 with water) was added to 2.5 ml of sodium carbonate solution (20%) in each tube. After Vortexing the test tubes were kept aside in dark for 40 min and the absorbance was measured at 725 nm against the blank solution. The analysis was performed in triplicate and the content of the phenolic compounds was expressed as mg gallic acid equivalent (GAE)/g sample.The extracts of the same was used to estimate the tannins with polyvinyl polypyrrolidone (PVPP) (Siddhuraju and Manian, 2007). 100 mg of PVPP was added to a 100×12 mm test tube, distilled water and sample extracts of 1ml in volume were added to the test tubes . The content was shaken well and kept at 4°C for 4h. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. The supernatant consisted of only phenolics not tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics (tannic acid equivalents) on a dry matter basis. From the above results, the tannins present in the sample were calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%)

Determination of total flavonoid content

The determination of the flavonoid content was performed according to the colorimetric assay described by Zhishen*et al*. (1999). 0.5ml aliquot of the diluted sample (10mg/2ml) was mixed with 2ml of distilled water and 0.15ml of 5% sodium nitrite solution. The reaction was allowed to progress for 6 min. After that 0.15 ml of 10% Aluminium chloride solution was added. After 6 min , 2ml of 4% sodium hydroxide

solution was added and the mixture was diluted and made up to 5 ml and then the reaction mixture was mixed and allowed to stand for another 15min. Absorbance at 510 nm was read immediately. The analysis was performed in triplicate and the results were expressed as rutin equivalent.Table-3

Table 3 Total phenolics, tannin and Flavonoid content

Constituents	Content
Total Phenolics (mg TAE/g extract)	92.40 ± 0.83
Flavonoid (mg RE/g extract)	1.17 ± 0.03
Total Tannin (mg TAE/g extract)	48.80 ± 0.83

Values are means of three independent analyses of the extract ± standard deviation (n = 3).

TAE – Tannic acid equivalent; RE – Rutin equivalent

RESULTS AND DISCUSSION

Alpinia galangal leaf extracts in solvents of different polarity were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria. Among the 5 types of solvents extract acetone (80%) showed highest antibacterial activity. Second most chloroform showed (64.2%) and followed by Petroleum Ether (28.5%), ethanol (8.5%). Our investigation showed that the Acetone extract exhibited a huge degree of antibacterial activity against burn, accident, skin infection, abscess, Trauma and post operative wound isolates as compared to chloroform extract. In this study no activity was observed from methanol extract. Among them highest activity accident wound isolates (85.7%), particularly *E.coli*was highly suppressed by acetone extract but same Pseudomonas aeroginosa was resistance to acetone extract.

The inhibition zone ranged from 13-26 mm was observed. The plant extract ranged from 5mg, 12.5mg and 25mg. The inhibition zone was started from 12.5mg concentration and size of zone (mm) ranges from 8 to 26mm, in the same time all isolates were inhibited by 25mg concentration and inhibition zone (mm) ranges from 8-35mm.

.When the results obtained were compared to those of standard antibiotic; plant extract was compared to those of standard antibiotic tested, this indicated that the extract when purified will be a high potent antimicrobial agent. Phytochemical tests are used to identify the chemical constituents in the plant material that have biological activities. The results of our preliminary phytochemical study revealed the presence of Saponins, Phenolic compounds, Alkaloids, Flavonoids, Glycosides, Starch, Phenols and Tannins, Carbohydrates, proteins and Amino Acids, Steroids & Sterols (Horbone (1998) and Kokate et al.,(1995) .The ethanolic extract contained the highest concentrations of totalenolic compounds (92.40 ± 0.83**(**mg TAE/g) and flavonoids (13.78 mg TAE/g) and Tannins 48.80 ± 0.83**(**mg TAE/g).

CONCLUSION

From the above experiment it can be seen that *A. galanga*leaf acetone extracts showed significant activity against Gram-positive and Gram-negative bacteria. The activity leaf was found to be quite comparable with the standard antibiotics screened under similar conditions. So the oil can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria. As the leaf acetone extract is found to be active against *E.Coli,Pseudomonasaeruginosa*and chloroform extract against *Proteus vulgaris*, and their activities are comparable with the standard antibiotics they can be used for the treatment of bacterial infections. The activity of the leaf extracts with acetone on *E.coli*was higher (85.7%) in Burn wound samples and Pseudomonas aeroginosa with 30 mm (MIC) in accident wound samples.On a whole result is as follows:

Acetone extract

Acetone extract showed antibacterial activity against burn, accident, skin infection, abscess, Trauma and post operative wound isolates. Among them highest activity accident wound isolates (85.7%), particularly *Pseudomonas aeruginosa* was highly suppressed by acetone extract but same time *E.coli* was highest in burn samples to acetone extract.

Table 1 Antibacterial Activity of *alpiniagalanga*leaf extract

Extract	Zone of inhibition (mm in diameter) 25 mg/ml					
	E. coli	***K. pneumoniae***	***P. aeruginosa***	***S. aureus***	***Proteus sps***	***Streptpyogenes***
Acetone	35	15	30	15	11	12
Chloroform	11	12	11	17	15	12
Ethanol	-	26	-	-	-	-
Methanol	-	-	-	-	-	-
Petroleum ether	-	-	-	11	-	12
Ampicillin	28	25	25	17	19	15

Among the test organisms used E.coli, Klebsiella pneumonia, Pseudomonas Aeruginosa, Staphylococcus. Aureus, Proteus sps, Streptococcus pyogenes.E.coli was the most susceptible bacteria to the acetone plant extract with 35 mm zone of inhibition in Burn wound samples followed by Pseudomonas aeroginosa with 30 mm (MIC) in accident wound samples

Chloroform extract

The highest antibacterial activity of Chloroform against to burn and trauma wound isolates (77.7%) followed by abscess wound isolates (75%), skin infection and post operative (50%). In percentage wise *Proteus vulgaris* were highly suppressed by chloroform extract but same to number of

Table 2 Preliminary Phytochemical Screening

Phyto constituent	Name of test	Presence/ Absence
Alkaloids	Wagner's test	+
Flavonoids	Lead acetate test	+
	Shinoda test	+
Saponins	Honey comb test	+
	Foam test	+
Phenols & Tannins	Lead acetate test	+
	Ferric chloride test	-
	Sodium hydroxide test	-
Carbohydrates	Fehling's test	+
	Benedict's test	+
Protein &Aminoacids	Biuret test	+
	Ninhydrin test	-
Steroids & Sterols	Salkowshi's test	+
Glycosides	Glycoside test	+

Note: "+"-Presence of compounds **"-"**- Absence of compounds

Pseudomonas aeruginosa was resistance to chloroform extract.

Methanol extract

In this studies no antimicrobial activity were observed from methanol extract. All wound isolates were resistance to methanol extract.

Petroleum ether extract

The highest antibacterial activities of Petroleum ether against accident wound isolates (35.7%). Among the 7 isolates, *Staphylococcus aureus*and *Streptococcus pyogenes* was highly suppressed by Petroleum ether extract.

Ethanol Extract

The highest antibacterial activities of ethanol extract against burn wound isolates (14.8%). Among the 7 isolates *Klebsiellapneumoniae* only suppressed by ethanol extract. The inhibition zone ranged from 13-26 mm was observed.As the leaf extract exhibited pronounced activity, essential oil from leaves into the drug formulations is recommended.As a result of high phenolic content that istheethanolic extract contained the highest concentrations of total phenolic compounds (92.40 ± 0.83(mg TAE/g) and flavonoids (13.78 mg TAE/g) and Tannins 48.80 ± 0.83**(**mg TAE/g) it might prove that it should possess natural antioxidant in food products.

References

1. BACIS-Boelens Aroma Chemical Information Service (CD-ROM), *Volatile Compounds in Food. (VCF) DATABASE*, *Alpinia* species, Zeist, (2000).
2. Chung, K.T., Thomason, W.R. and Wu-Tuan, in C.D (1990). Growth inhibition of selected food borne bacterial particularia Listeria monocytogenes by plate extracts. *J. Appl. Bacteriology*. 69,498-509,
3. Cote, R.J. and Ghernal, R (1994). Nutrition and Methods for General and Molecular Bacteriology, 2nd Edn, by P Gernardt (Ed), *American Society for Microbiology*, Washington DC.155-178.
4. Cheesebrough, M. (2004). District Laboratory Practice in Tropical Countries part 1 2nd edition Low price Edition, Cambridge, Pp340-349.
5. Cowan ST (1985). Cowan and Steel's for the identification of Medical Bacteria, 3rd ed. Cambridge University press, London, pp. 81-100.
6. Fawole MO, Oso BA (1988). Laboratory Manual for Microbiology, I st Edition, Spectrum Book Ltd, Ibadan. Pp. 22-45.
7. *Handbook of Medicinal Herbs*(2nd ed.). Boca Raton, FL: CRC Press. p. 350. ISBN 0-8493-1284- 1. Retrieved 1 March 2011.
8. HorboneJB(1998). Phytochemical methods, a guide to modern techniques of plant analysis. Edition - 3.london: Chapman and Hall. 42-278.
9. Kokate CK. (1986). In: Preliminary phytochemical screening, Practical Pharmacognosy, Ist ed., Vallabh
10. Prakashan, New Delhi, pp111.
11. Mohana DC, Satish S. and Raveesha KA (2008). Antibacterial evaluation of some plant extracts against
12. some human pathogenic bacteria. *Advances in biological Research.*2 (3-4):49-55.
13. J. Oonmetta-aree, T. Suzuki, P. Gasaluck and G. Eumkeb.(2006). *Food Science and Technology*, 39(10), 1214.
14. Shivkanya. J, Nitin.I, .Dinesh.S.Neeraj.F and Mohamad(2009).A., *Int J Green Pharm*,3 (2), 144.
15. 13. Siddhuraju P, Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents
16. from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric. Food.
17. Chem.51, 2144-2155.
18. Siddhuraju, P. and Manian, S (2007). The antioxidant activity and free radical scavenging capacity of dietary
19. phenolic extracts from horse gram (Macrotylomauniflorum (Lam.) Verdc.) seeds. Food Chemistry 105, 950-

21. 958 (Elsevier, UK).
22. Thakur, R. S., Puri, H. S. and Husain, A. 1989. *Major Medicinal Plants of India*, CIMAP, Lucknow,
23. India.pp.50-52.
24. V. Vudhakul, P.Bhoopong,F. Hayeebilan and S.Subhadhirasakul, (2007). *Food Microbiology*, 24(4), 413.
25. Yang, X. and R.G. Eilerman. (1999). Pungent principle of *Alpinia galangal* (L.)Swartz and its application.*J.*
26. *Agric. Food Chem*. 47:1657-1662
27. Zhishen J, Mengcheng T, Jianming W (1999). Research on antioxidant activity of flavonoids from natural
28. Materials. Food Chem. 64:555-559.
29. Zheng, G.Q., P.M. Kenney and L.K.T. Lam (1993).Potential anti carcinogenic natural products isolated from
30. Lemon grass oil and galanga root oil. *J. Agric. Food*.41: 153-156.

****