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ANTIMICROBIAL, ANTIOXIDANT, ANTIINFLAMMATORY ANALYSIS OF ACTIVITY AND PHYTOCHE MICAL SCRE E NING OF CINNAMOMUM VERUM, ACOROUS CALAMUS, CINNAMOMUM CASSIA, G LYCERIA G LABRA

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ABSTRACT

Cinnamon is a spice obtained from the inner bark of several tree species of the genus cinnamomum. Cassia is the Chinese cinnamon spice consisting of aromatic bark of the cinnamomum cassia plant of the family Lauraceae. Acorus calamus is also called sweet flag (vasambu). The root has a lot of medicinal properties. G lyceria glabra (glycyrrhiza) is a medicinal species also called Adhimathiram in Tamil. These are taken as samples to the present study on evaluation of antimicrobial, antiinflammatory, antioxidant activities and phytochemical analysis. The samples are dried and powdered with methanolic solution. The phytochemical analysis of these four samples shows the presence of many bioactive compounds like carbohydrates, flavonoids, proteins, aminoacids, tannins and steroids. It shows effective and determinative antimicrobial activity against the bacterial strain of staphylococcus aureus and is done using agar well diffusion method, forms a zone of inhibition. The antioxidant activity is done by using DPPH free radical scavenging method and shows effective scavenging activity in these four different samples. The antiinflammatory activity has been performed by using inhibition of the protein denaturation method (egg albumin). It shows maximum absorbance in the samples. Therefore, these samples show better results and are useful in the future for designing drugs that present all herbal and medicinal activities.

KEYWORDS: Antimicrobial activity, Antioxidant activity, Antiinflammatory activity,

Phytochemical screening, Staphylococcus aureus.

INTRODUCTION

Cinnamon is used mainly as an aromatic condiment and flavouring additive in a wide variety of cuisines, sweet and savoury dishes, breakfast cereals, snackfoods, tea and traditional foods. The aroma and flavour of cinnamon derive from its essential oil and principal component, of Cinnamaldehyde as well as numerous agar constituents, including Eugenol. All are members of the genus cinnamomum in the family Lauraceae. Only a few cinnamomum species are grown commercially for spice. Cinnamomum verum is sometimes considered to be "true cinnamon", but most cinnamon in international commerce is derived from related species, also referred to as "cassia". In 2016, Indonesia and China produced 75% of the world' s supply of cinnamon. Chinese cassia or Chinese cinnamon is an evergreen tree originating in Southern Asia and widely cultivated in southern and eastern Asia. The buds are also used as spice, especially in India, and were once used by the ancient Romans. The tree grows to 10-15 meters tall. Due to a blood- thinning component called Coumarin, which could damage the liver if taken in huge amounts, Europian health agencies warned against consuming high amounts of cassia. In high doses these substances can also be toxic to humans. Acorus calamus is a plant used by medicinal agencies during seasoning and the herb traditionally use in Ayurveda and traditional Chinese medicine for its cognitive- enhancing properties. It contains beta- asarone, which is toxic and a known carcinogen and it also responsible for the plants main mechanism though it is possible to extract and remove beta- asarone from the plant in order to make supplementation safe. G lycyrrhiza is a genus of about 20 accepted species in the legume family. It is a liquorice plant which is an herbaceous perennial legume native to southern Europe and parts of Asia, such as India.

These different samples contain antioxidant compounds like flavonoids, carotinoids and polyphenols and possess antiinflammatory, antimicrobial potential. Chemical substances derived from medicinal plants that produce a definite physiological action on the human body are called phytochemicals and are a rich source of secondary metabolites.[1,2] The plant constituents with antioxidant activity are capable of exerting protective effects on biological system and they contains antioxidants which report on medicinal plants with antioxidant potential.[3,4] Traditional medicines for arbotifacients, contraceptives, menstrual regulation, fertility control have been used by these plant extracts.[5] The stimulating adaptive immune system is enhanced by antimicrobial protein which produces species including bacteria.[6] The

antimicrobial activity are also known as anti- helminthic activity, detoxifier, immunity booster and has anti- parasitic activity.[7] Biological functions of proteins are denatured by the antiinflammatory activity induced by carrageenan and the drugs used for pain management and inflammatory conditions have toxic side effects when administrated clinically.[8,9,10] This study describes a four different types of herbal sources used and they belong to Lauraceae family. These herbal plant sources have the property of a substance that reduces inflammation or swelling.

MATE RIAL AND ME THODS

Materials: Different herbal plant sources such as cinnamomum verum, Acorouscalamus, Cinnamomum cassia and G lyceria glabra were collected in Chennai (Tamilnadu) from a local market.

METHOD

Preparing the extract: 100g of each sample is taken individually and cut into small pieces. Then 50 g of different samples were powdered (coarse) using a mechanical grinder or mixers.10g of sample extracted with 100 ml of methanol was collected in a conical flask and incubated in a rotary shaker for 24hours. After 24 hours, the extracts were filtered with W hattman filter paper. The collected solvents were then evaporated to dryness using a drying oven incubator at 60ºC. The dried extract was powdered to get 10mg per ml concentration of extract.

Bacterial culture: Staphylococcus aureus collected from Apex Biotechnology Research and Training Institute, Chennai, has been used for antimicrobial activity.

Phytochemical screening: Q ualitative phytochemical tests are carried out with the methanol extracts of four different samples.

Test for carbohydrates: Benedict' s reagent- 1ml of extract is mixed with the few drops of Benedict' s reagent (alkaline solvent containing cupric citrate complex) and boiled in water bath. Formation of red or pink colour shows presence/absence of carbohydrates.

Test for anthraquinones Borntrager' s test

About 0.1g of each portion to be tested is mixed with 5ml of the 10% ammonium solution is then added to the filtrate and there after shaken. Apperanace of a pink, red or violet colour in the Ammoniacal (lower) phase was taken as the presence\absence of Anthraquinones.

Test for flavonoids

0.1 g of plant extract is boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution are then added. A green- blue or violet colouration indicated the presence of a phenolic of hydrolic group and it shows the presence/absence of flavonoids.

Test for proteins Biuret test: Take 0.2g of sample added with 1ml of distilled water heated with 10% of sodium hydroxide solution and 2 drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour which shows presence of proteins.

Test for free aminoacids Ninhydrin test

Test solution when boiled with 0.25% solution of Ninhydrin would result in the formation of purple colour suggesting the presence/absence of free aminoacids. Test for coumarin.

To 1ml of test solution, a few drops of alcoholic sodium hydroxide has been added. Appearance of yellow colour indicates the presence/absence of coumarin.

Test for saponins

200 mg of extract is boiled with 3ml of distilled water, filtered. To the filtrate, about 3ml of distilled water is further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming is taken as an evidence for the presence/absence of saponins.

Test for steroids: 0.1 g of each portion, 2ml of acetic acid is added, the solution is cooled well in ice followed by the addition of concentrated sulphuric acid carefully. Colour change from violet to blue or bluish- green indicated the presence of a steroidal ring. i.e, a glycone portion of cardiac glycoside.

Test for tannins: 0.1g of each portion was stirred with about 2ml of distilled water and then filtered. Few drops of 1% ferric chloride solution has been added to 2 ml of the filtrate. Appearance of a blue- black green or blue- green precipitate indicates the presence of tannins.

E stimation of total phenol

The solvent extracts are used for the determination of the total- phenolics by Spectrophotometrically by Folin- Ciocalteau colorimetric method. Each extract (200µl) was added into a screw cap test tube and 1ml of Folin- Ciocalteau (1:1 with water) and 1ml of sodium carbonate (7.5%) were added. The mixture was vortexed, incubated for 2hours and

the absorbance was read at 726nm using a spectrophotometer. The total phenolic content was expressed as G allic acid Equalance (G AE) in mg\g dry material. Total phenol is calculated by, Total phenol= (Control OD – Test OD) / Control OD × 100

Antimicrobial activity test

The antimicrobial activity test was done using the Agarwell diffusion method to detect the presence of antibacterial or antifungal property of the extracted samples. A nutrient agar is prepared and poured into a sterilized petridish. Sterile swabs were used to distribute the bacterial culture (Staphylococcus aureus) evenly over the surface of the nutrient agar plates and the test samples were introduced into the wells at different concentrations varying from (25,50,75,100µg\ml). The antibiotics streptomycin is introduced into the centre of the well for control. Then the plates are labelled and incubated overnight at 37ºC. Microbial growth is determined by measuring the diameter of zone of inhibition and it is measured in mm.

Antioxidant activity test: The antioxidant activity test is done by using the DPPH(2,2-Diphenyl- 1- picrylhydraczyl) free radical scavenging assay. According to the procedure of Von G adow et al (1977), 2 ml of 6 × 10- 5 µ methanolic solution of DPPH is added to a 50 µl of methanolic solution of sample at a concentration of 10mg\ml. Then, the samples were incubated for 16minutes at room temperature in a dark place. The blank is adjusted with methanol in DPPH for obtaining value of 0.629 and kept as control and the absorbance was noted at 515nm using a UV- spectrophotometer. The percentage inhibition of DPPH radical of the samples were calculated according to the formula of yen and Duh (1994).

IP = AC(0) – AA(t) / AC(0) × 100

Where,

AC(0) is the absorbance of control at t = 0 min; and AA(t) is the absorbance of antioxidant at t = 16 minutes. Antiinflammatory activity test.

The antiinflammatory activity was done using the inhibition of protein denaturation method. The reaction mixture consists of a 100 µl egg albumin(white yolk from fresh hen’ s egg), 9.9ml double distilled water, 20 µl of concentrated hydrochloric acid and 25 µl of sample extracts. The double distilled water is used as a blank. Then the samples are incubated at 37ºC for 20 minutes and 57ºC for 30 minutes. After cooling, the absorbance of sample is measured at 660nm in a spectrophotometer. The percentage inhibition of protein denaturation was calculated by using equation,

Percentage inhibition = Abs Control – Abs Sample / Abs Control × 100

RE SULTS AND DISCUSSION

Rendemen value

Table 1: Results of Phytochemical screening of extracts.

Chemical content	Cinnamomum verum	Acorous calamus	Cinnamomum cassia	G lyceria glabra
Carbohydrates	+	+	+	+
Anthroquinone	-	-	-	-
Flavonoids	-	-	-	+
Proteins	-	-	-	-
Aminoacids	-	+	-	-
Coumarin	-	-	-	+
Saponins	-	-	-	-
Tannins	-	-	-	+
Steroids	-	-	-	-

(+) positive reaction (-) negative reaction

The qualitative phytochemical analysis of the four different samples showed the presence of carbohydrates, flavonoids, aminoacids, coumarin and tannin and absence of protein, anthraquinone, saponins are shown in Table 1. The absence of saponins shows the action as antibacterial mechanism resulting in leakage of intracellular cells and lead compounds.

Table II. Results of total phenolic content of extracts.

Extract	Total phenolic content
Cinnamomum verum	0.912
Acorous calamus	0.712
Cinnamomum cassia	0.455
G lyceria glabra	1.557

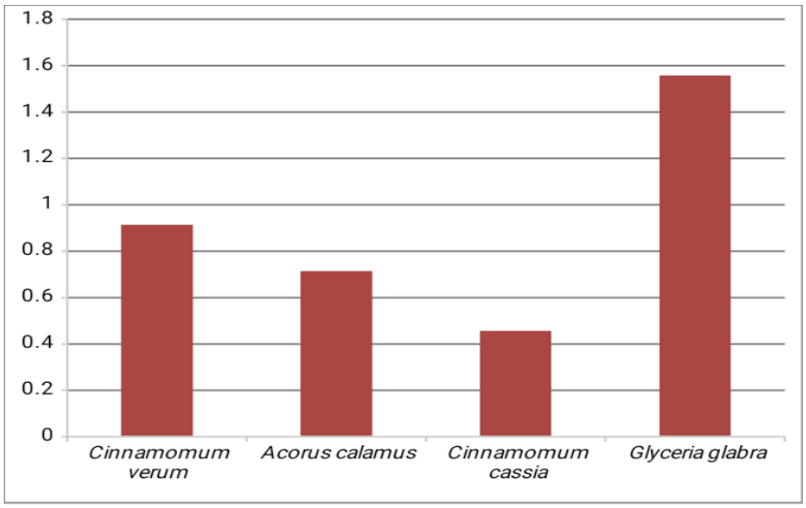

Figure. 1: The Results total phenolic content of samples (extract) Cinnamomum verum, Acorus calamus, Cinnamomum cassia, G lyceria glabra.

Figure 1 showed the total phenolic content of methanolic extract for four different herbal samples and the higher total phenolics content were observed in Cinnamomum verum, Acorous calamus, Cinnamomum cassia and G lyceria glabra and the results are shown in table 2.

G lyceria glabra > Cinnamomum verum > Acorous calamus > Cinnamomum cassia The phenolic compounds have high redox potential which allow the extract to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Figure 1 shows the phenolic content of extracts from higher to lower. G lyceria glabra has the higher phenolic content when compared with the other sample extracts.

Table. III. Results of antimicrobial activity test of extracts.

E xtract	Concentration (µg/ml)				Cont rol
	25	50	75	100	
Cinnamomum verum	**0**	**0**	**6**	**4**	**9**
Acorous calamus	**0**	**0**	**2**	**1.5**	**7**
Cinnamomum cassia	**0**	**1**	**5**	**5**	**9.5**
G lyceria glabra	**0**	**2**	**3.5**	**6**	**8**

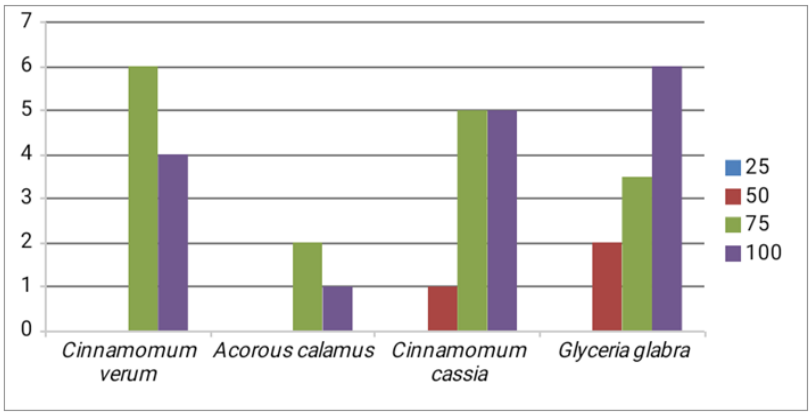

Figure. 2: The diameter of inhibition zone of samples (extract and concentration variable) on Cinnamomum verum, Acorous calamus, Cinnamomum cassia, G lyceria glabra.

Figure 2, the antimicrobial activity of the extracted samples at different intensity and specificity. The samples shows effective and determinative antimicrobial activity against the bacterial strain of staphylococcus aureus. 75 µg/ml and 100 µg\ml show similar result of zone of inhibition. On comparing the zone of inhibition, the sample extracts show a higher zone than the other. The inhibition zone of Staphylococcus aureus bacteria measure (25%, 50%, 75%, 100%).

Table. IV. Results of antioxidant activity test of extracts.

E xtract	Antioxidant activity
Cinnamomum verum	0.392
Acorous calamus	0.463
Cinnamomum cassia	0.446
G lyceria glabra	0.430

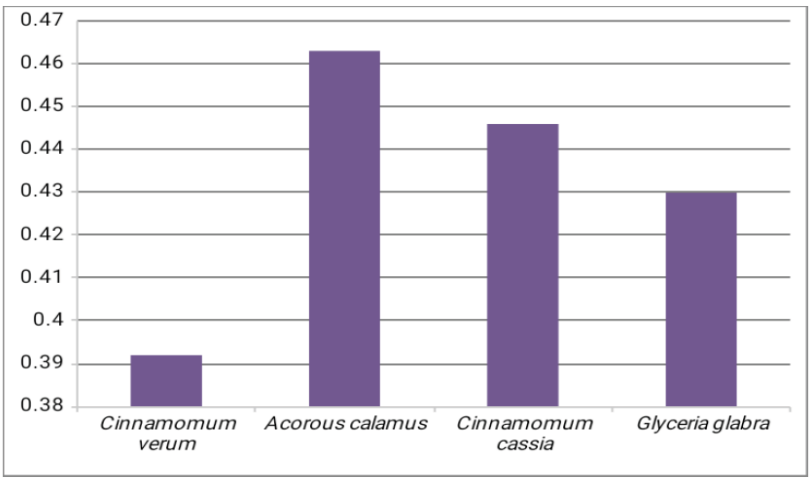

Figure. 3: Antioxidant activity of samples (extract) Cinnamomum verum, Acorous calamus, Cinnamomum cassia, G lyceria glabra.

The methanol extracts of these different samples exhibit antioxidant activity. The sample Cinnamomum verum shows a lower radical scavenging activity and effective in the methanol extracts (0.392µg/ml). And sample Acorous calamus containing (sweet flag) has higher value (0.463µg\ml) W hich indicates higher scavenging activity. G lyceria glabra also contains a higher value (0.430µg/ml) and has activity of free radical scavenging.

Table. V. Results of anti- inflammatory activity test of extracts.

E xtract	Antiinflammatory activity
Cinnamomum verum	1.259
Acorous calamus	1.246
Cinnamomum cassia	0.942
G lyceria glabra	0.778

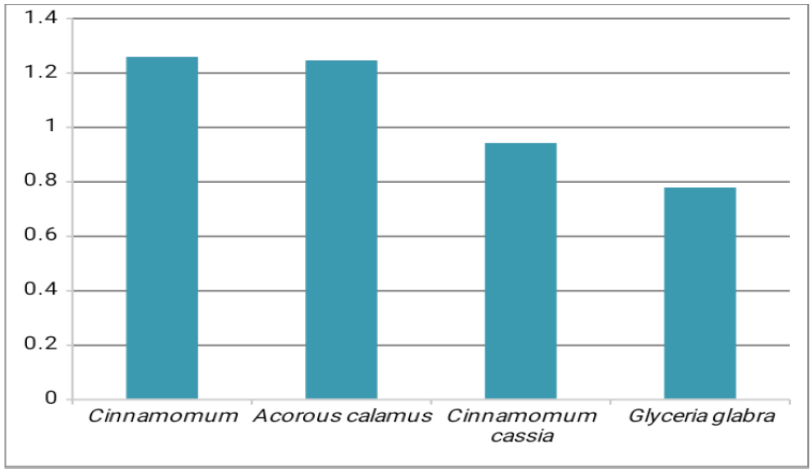

Figure. 4: Antiinflammatory activity ofsamples (extract) Cinnamomum verum, Acorous calamus, Cinnamomum cassia, G lyceria glabra.

Protein denaturation is a loss of biological properties of protein molecules. The present study shows the anti- inflammatory activity of methanolic extract of different samples on inhibiting denaturation of proteins as shown in the Table 4. The maximum inhibition observed in sample Cinnamomum verum (cinnammon) is (1.259 µg\ml) and the minimum inhibition of (0.778 µg\ml) is observed in sample G lyceria glabra (glycryrrhiza). The change in colour indicates the protein denaturation in the sample. Changes in pH affect the amino acid residues of samples and lead to denaturation and hydrogen bonding also involves in these side changes.

DISCUSSION

In the present study, the results of extract shows the reducing capacity of compounds that serve as potential indicator for antioxidant properties. The phytochemicals like flavonoids and tannins present in plant species that belonging to Leguminosae - Mimiosoideae family.[1,2]

Free radicals due to their scavenging activity involve in management of diseases like neurodegenerative problems, cancer, etc. Ascorbic acid involved in the formation of free radicals and formation of intercellular substances.[3,4,5]

Further studies reported that the level of effectiveness are compared with other samples in antimicrobial activity. The samples shows no activity against the interaction with gram negative bacteria, some resistance to the waterborne pathogens. The temperature treatment of extracts gives the potency of samples by thermal degradation for phyto – constituents, this

process is called thermostability.[6,7] Presence of polyphenolic compounds, flavonoids, terpenoids and free radical inhibitors shows the inflammatory inhibition. According to T.Dimo et al formalin in the extracts induces acute inflammation that involves the production of endogenous mediators.[8,9,10]

CONCLUSION

1. The present study reveals the presence of many phytochemical compounds like carbohydrates, flavonoids, aminoacids, coumarin and tannins.
2. The four different herbal samples extracted exhibit the antimicrobial, antioxidant and anti-inflammatory potential. These sources show better results in all activities.

They lead a good role for dietary, energy metabolism and regulate blood pressure, cardiac function etc and in future will help designing new drugs that will provide sufficient energy effectively.

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REFERENCES

1. Padmanabhan, Vasanthi, Manimekalai, G anapathy, Vasthi Kennedy Evanjelene, Nirmala Ayyavu, Jagajothi Angamuthu. Phytochemical screening and antioxidant activity of extracts of the leaf and bark of Albizzia lebbeck (benth). Academic journal of medicinal plants, 2(2): 026- 031.
2. M.U.G hurde, S.N.Malode. Phytochemical Screening and Arresment of Biomolecules Compounds in Scilla hyacinthine (roth) Macbr. BULB. Journal of G lobal Biosciences, 2014; 3(5): 866- 871.
3. Prakash Veeru, Mishra Pankaj Kishor and Mishra Meenakshi. Screening of Medicinal Plant Extracts for Antioxidant Activity. Journal of Medicinal Plants Rresearch, 3(8): 608- 612.
4. Rajendra Kshirsagar, Shakti Vpadhyay. Free Radical Scavenging Activity Screening of Medicinal Plants from Tripura, Norteast India. Natural product, Radiance, 2009; 8(2): 117-122.
5. K.O.Akinyemi, O.K.Oluwa, E.O.Omomigbehin. Antimicrobial Activity of Crude Extracts

of Three Medicinal Plants used in South- West Nigerian Folk Medicine on some food borne Bacterial Pathogens. Afr. J. Trad, 2006; 3(4): 13-22.

6. Ashwini Kasliwal and Farha quadei. Evaluation of Antibacterial Activity of Punica granatum. Journal of G lobal Biosciences, 2016; 5(1): 3534-3538.
7. Onsare JG, Kaur H, Arora DS. Antimicrobial Activity of Moringa oleifera from different locations against some human pathogens, Academia Journal of Medicinal Plants, May 2013; 1(5): 080-091.
8. M.V.Anoop, A.R.Bindu. In- vitro antiinflammatory activity studies on Syzygium zeylancium (L.) DC leaves. International Journal of pharma research & review, August 2015; 4(8): 18- 27.
9. T.Dimo, Agathel L- fotio, T.B. Nguelefack, E.A.Asongalem, P.Kamtchouing. Antiinflammatory activity of leaf extracts of Kalanchoe crenata ande. Indian Journal of Pharmacology, Febrauary, 2006; 38(1).
10. TS Divya, PG Latha, Kusha, G J Anuja, SR Suja, S Shyamal, VJ Shine, S Sini, P Shikha and S Rajasekharan. Antiinflammatory, Analgesic and Anti- lipid Peroxidative properties of Wattakka volubilis (Linn.f.) stapf. Natural Product Radiance, 2009; 8(2): 137-141.
11. j. patel chirag, Satyanand Tyagi, Nirmala Halligudi, Jaya Yadav, Sacgcgidanand Pathak, Satya Prakash Singh, Ashish Pandey, Darshan Singh Kamboj, Pratap Shankar. Antioxidant Activity of Herbal Plants. Journal of Drug Discovery and Therapeutics, 2013; 1(8): 01-08.