



PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY POWER OF ANNAPATHI CHENDURAM

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ABSTRACT

Annabathi chenduram is a poly herbal and used for medicine various forms such as chooranam. The plant is one of the chief constituents used for various preparations of siddha medicine. My research since 1985, with the addition of *Citrus medica*, *Calotropis gigantia*, *Rhinacanthus nasutus* and *Amaranthus gangetious* nutrient delivery medicine in addition to A.chenduram. The present study to investigate phytochemicals present in the A.chenduram" contain phytoconstituents like alkaloids, tannins, flavonoids, phenols, saponin and cardiac glycosides. GC-MS analysis of yielded 9 peaks showing the presence as nine compounds (Hexadecanoic acid, 4H-1-Benzopyran-4-one, Phytol, Octadecanoic acid, Oleic acid, Eicosanoic acid, Flavone, Docosanoic acid, Docosanoic acid,3,4-dimehyl). These results antibacterial activity revealed that A. chenduram chooranam showed a good inhibitory activity against human pathogens (*Bacillus subtilis*, *Staphylococcus*, *Escherichia coli*, *Klebsiella pneumonia*). The phytochemical have a strong role like anti tumor, Anti oxidant, anti-inflammatory, anticancer, antiviral, antibacterial and analgesic properties.

KEYWORDS: Phytochemical screening: GC-MS, antioxidant and antibacterial activity.

1. INTRODUCTION

Medicinal plants have been used therapeutically all around the world and is an important aspect of various traditional medicine systems. From Ayurveda to the Chinese traditional medicine from Unani to Tibetan medicine from Amazonian to African medicine all systems although based on different theoretical and cultural models to integrate the phytotherapy into their doctrine (WHO, 2007). Medicinal systems such as Ayurveda, Yoga, Unani, Siddha and other traditional system are in regular practice all over India, and more so in rural areas. All these systems used plants and different parts of medicinal plants as the main ingredients of the medicine.

Ayurvedic drugs are comprises of the standardization of single drugs, finished product and method of manufacture standardization of method of manufacture includes the department of some modern economic, convenient method us manufacture without prejudice to its therapeutic value either by modifying. The standardization also helps to give scientific explanation to the basic principles of ayurvedic science and to reduce the cost of production of products to meet the needs of poor people, besides this such a study will help the development, program and propagation of ayurveda and outside our country this proper deals with the

modification in the methods of manufacture of "*Annabathi chenduram*".

A large and ever expanding global population base that prefers the use of natural products in treating and preventing various medical problems because herbal plants have proved to have a rich resource of medicinal properties (Retnam and De Brito 2003). Many commercially proven drugs used in modern medicine were initially med in crude from in traditional or folk healing practices or for other purposes that suggested potentially useful to biological activity (Balandrin et al., 1988).

Microorganisms are the causative agents of almost all kinds of acute and chronic diseases antibacterial have enormous therapeutic potential. plant extracts with known antibacterial properties can be at great significance in therapeutic treatments. Although hundreds of plant species have been tested for antibacterial properties, the majority of them have not been adequately evaluated (Harborne et al., 1998) considering the vast potentiality of plants as sources for antibacterial drugs with reference to antibacterial agent, a systematic investigation was under taken to screen by poly herbal formulation Annabathi chenduram for antibacterial activity against selected human pathogens.

Therefore followed by with further research I have used this green vitrol [power Annapathi chenduram] to heal different types of unknown fever that kills people in many places of our country. So in upcoming day's this medicinal plant can be used to treat bird flue, chickenkuniya even it can cure joint arthritis as it accures because of some nutrient deficiency that will give best relief for our future generation.

2. MATERIALS AND METHOD

2.1 Collection of plant

The free healthy and disease and fresh leaves of *Rhinacanthus nasutus*, *Amaranthus gangeticus*, *Calotropis gigantea*, *Citrus medica* and *Mentha* (Figure-1) was collected and in around the Kanchipuram, Tamilnadu, India during the months of December 2014 and January 2015. After that the fresh leaves was pulverized into Juice. 100 ml juice of leaves was then filtered and stored. The stored extract of juice was collected as well as its yield was also measured.

2.2 Preparation of Annapathi chenduram

Annapathi (Green vitrol) is been dissolved in water and it is boiled till 100°C then it have been chilled so that it becomes crystals and these crystals weight of 350 grams is been mixed and grind with 100 ml of *Citrus medica* (lime) extract and it is dried then 10 kg of varati (dried cow dung) is been burnt with the dried and the burnt ashes is been mixed with 100 ml of *Calotropis gigantea* extract, 100 ml of *Amaranthus gangeticus* extract, 100 ml of *Rhinacanthus nasutus* extract and 100 ml of mentha extracts is been added. This mix is dried again and it is been burnt with 10 kg of varati (dried cow dung) the ashes is stored in the earthen pot preserved under the soil for two to three days and after that the final product red ashes is collected. The red ashes is used for medical purposes and for further analysis.

2.3 Qualitative and quantitative analysis

The qualitative and quantitative analysis were carried out A. chenduram using standard procedures (Shrivestav et al., 2009).

2.4 In vitro antibacterial activity

2.4.1 Selection of the microorganisms

The bacterial strains used for this study *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 23857), *Klebsiella pneumonia* (ATCC 31488), *Staphylococcus aureus* (ATCC 25923), All the stock cultures were obtained from American Type of Culture Collection (ATCC, Manassas, USA).

2.4.2 Well Diffusion method

2.4.3 Preparation of inoculums

The inoculum size of the test isolates was standardized according to the National Committee for Clinical Laboratory Standards guidelines (CLSI, 2009). The bacterial strains were inoculated in Muller Hinton Broth [Hi-media, Mumbai, India] and incubated at 37°C in a shaker water bath for 3-6 h until the culture attained a

turbidity of 0.5 McFarland Unit. The final inoculums size was adjusted to 5×10^8 CFU/ml.

2.5 DPPH radical Scavenging activity of A.chenduram

For the evaluation of the free radical scavenging capacity, the DPPH (2,2-diphenyl-1, picryl hydrazyl) assay was used. Briefly, 50µL aliquots of various concentration of the sample [20,40,60,80,100,120 and 140 µg/ml] were added to 5 ml of 0.004% methanol solution of DPPH freshly prepared. After 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm with Gallic acid as a reference standard in a DU 40 Spectrophotometer (Beckman, US). Decrease in the DPPH radical scavenging activity. All the determinations were carried out in triplicates. This activity is given as percentage DPPH radical scavenging, which is calculated with the equation:

$$\text{Radical Scavenging (\%)} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})}{(\text{Absorbance}_{\text{control}})} \times 100$$

2.6 GC-MS Analysis of A. chenduram

The Annapathi chenduram was analyzed through gas chromatography and mass spectroscopy (GC-MS). The components of plant extracts were identified by comparison of their relative retention times and the mass spectra with those authentic reference compound and by computer matching of their MS spectra with, NIST 8 and Fame mass spectral library.

3. RESULTS AND DISCUSSION

3.1 Qualitative and quantitative phytochemical screening of A. chenduram

The preliminary phytochemicals screening of Annapathi chenduram revealed the presence of alkaloids (10.32mg), tannins (1.36mg), flavonoids (5.3mg), cardiac glycoside (1.985mg) steroids absent and saponnin (3.69mg) (Table -1).

Natural products are main source of synthetic and traditional herbal medicines. Medicinal plants are these which posses specific substance that can be used as therapeutics. Herbal drugs are easily available, cheaper, low cost and are safer then synthetic drug. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides and saponin (Bhandary et al., 2012). Based on the evaluation it can be accepted that the presence bioactive phytocomponents possess a good medicinal value.

The medicinal values possed by various phytochemicals increases the efficacy the A.chenduram like alkaloids can be used for treating cough and has anti-tumour, analgastic property, Steroids contains anti-inflammatory activity, Cardiac glycosides can be med in the treatment of cardiac activity Akiyama et al., (2001) whereas flavonoids possess anti-oxidant activity, anticancer activity Manashi et al., (1999) there bioactive

compounds are found in medicinal plants parts which are precursors for the synthesis of useful drugs.

3.2 DPPH radical scavenging activity of *A.chenduram*

DPPH radical scavenging activity of *A. chenduram* was increased with an increase in the concentration of *A. chenduram* (Figure -2). At a concentration of 1000 µg/ml, *A. chenduram* exhibited the highest and excellent percentage scavenging activity among the other four concentration of the *A. chenduram* (82.21%). while the 75µg of the *A. chenduram* yielded the lowest (29.12%). The IC₅₀ value of DPPH were found to be good (625 µg/ml) for 75µg concentration of *A. chenduram* than the other concentration of the *A. chenduram*. IC₅₀ values of *A. chenduram* of medicine were shown were based on the scavenging activity. Highest percentage of inhibition was obtained in 75µg of concentration followed by other concentration; 75µg has comparable scavenging activity with standard ascorbic acid (97.48%).

A.chenduram are good sources of antioxidants that serve as important nutraceuticals preventing oxidative damage (Alisi et al., 2011). The antioxidants needed by the human body to complement the exogenously produced ones are generally gotten from plants such as vegetables, herbs and fruits (Kordali et al., 2005).

3.3 GC-MS Analysis

The composition and identification of the main compounds present in the *A. chenduram* is shown in (Table-2). Nine compounds were hexadecanoic acid, eicosanoic acid, flavone, phytol, octadecanoic acid and oleic acid. The presence of different secondary metabolites in the *A. Chenduram* of this drugs shows that the drugs has much medicinal potential. Hexadecanoic acid, the most abundant compound (Figure -3) in the *A. Chenduram* has antioxidant properties (Devi et al., 2012). Phytol is a major cyclic diterpene alcohol and a precursor for vitamin E and K, It is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Kumar et al., 2010).

Hexadecanoic acid was also found to be present in the *A.chenduram*. In hexadecanoic acid are used in the treatment of rheumatism and inflammation (Aparna et al., 2012) The saponin has been shown to protect the fibroblasts cells from damage by hydrogen peroxide (Yoshikoshi et al., 1996).

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3.4 Antibacterial activity of *A.chenduram*

Antibacterial activity of "*A.chenduram*" was tested against laboratory test organism of biological importance *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas pneumonia*, The antibacterial potential as "*A. chenduram*" is well known. *A. chenduram* were tested for their antibacterial action (Table -4). The "*A. chenduram*" showed a higher antibacterial activity than the different concentration.

The diameters of the inhibition zones against all the tested bacteria were measured when tested by the disc diffusion method, the *A. chenduram* should show significant activity against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus* (Figure -4).

The contribution of multiple herbs has increased the efficacy of the *A.chenduram* to a greater extent, since they possess medicinal values excreted by the presence of phytochemicals. Flavonoids are known to be synthesized by plants in response to microbial infection. Hence it should not be surprising that they have been found to be effective as an antibacterial substance against a wide array of infectious agents (Prasad et al., 2008).

Tannins are also known as antibacterial agents they are water-soluble polyphenols and precipitated proteins present in many plant foods. Tannins have been reported to prevent the development of microorganism by precipitating microbial proteins. The growth of many fungi, yeasts, bacteria and viruses were inhibited by this compound (Nithya et al., 2012).



Figure 1. A). *Rhinacanthus nasutus*, B). *Amaranthus gangeticus*, C). *Calotropis gigantea*, D). *Citrus medica*, E). *Mentha*.

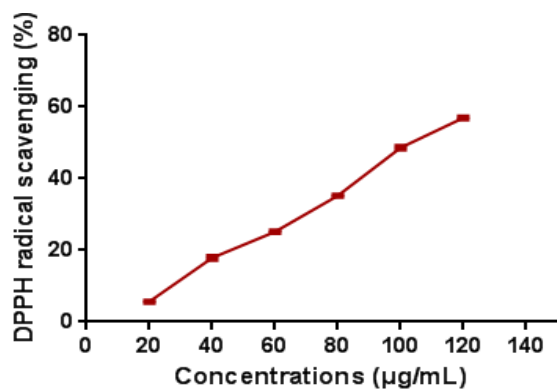


Figure 2: DPPH radical scavenging activity of Annapathi chenduram.

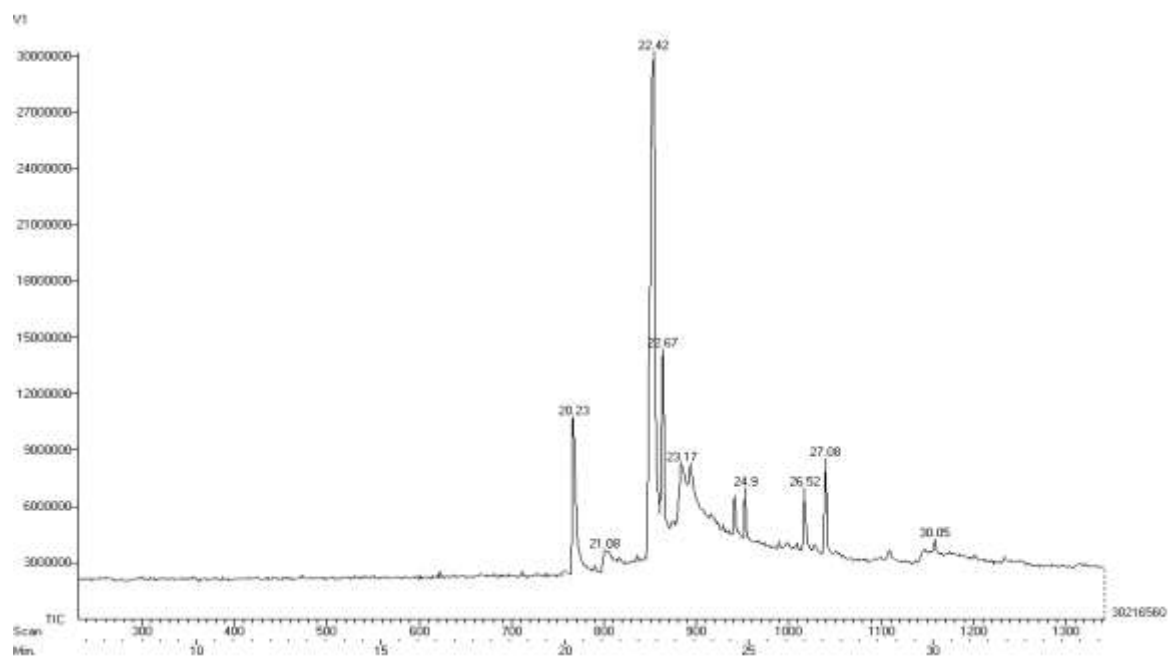


Figure 3 GC-MS analysis of Annapathi chenduram.



Figure 4: Antibacterial activity of Anapathi chenthuram.

Table 1: Phytochemical screening of Annapathi chenduram.

S.No	Phytochemical	Yield %
1	Alkaloids	10.32mg
2	Flavonoids	5.36mg
3	Saponin	3.69mg
4	Terpenoids	6.37mg
5	Glycosides	1.985mg
6	Tannins	1.36mg
7	Carbohydrates	1.34g
8	Coumarin	0.115mg
9	Protein	28.9009
10	Arginine	1.352
11	Phenols	0.63mg
12	Steroids	Absent

Table 2: GC-MS analysis of Annapathi chenduram.

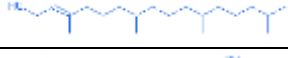
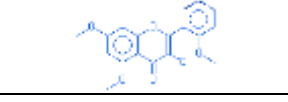
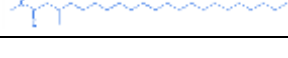
Sl.No	Retention Time	Area (%)	Compounds	Molecular formula	Molecular Weight	Structure
1.	20.23	36857632	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	
2.	21.08	6160768	4H-1-Benzopyran-4-one	C ₉ H ₆ O ₂	146.15	
3.	22.42	167615736	Phytol	C ₂₀ H ₄₀ O ₂	296.54	
4.	22.67	16761570	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.47	
5.	23.17	18968968	Oleic acid	C ₁₈ H ₃₄ O ₂	282.46	
6.	24.90	5324804	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312.53	
7.	26.52	10048568	Flavone	C ₁₅ H ₁₀ O ₂	222.24	
8.	27.08	11057876	Docosanoic acid	C ₂₂ H ₄₄ O ₂	340.58	
9.	30.05	4975868	Docosanoic acid,3,4,-dimethyl	C ₂₅ H ₅₀ O ₂	382.66	

Table 3: Activity of phyto-components identified in medicine of Annapathi chenduram by GC-MS analysis.

Sl.No	Name of the compound	Compound medicinal activities
1.	Hexadecanoic acid [or] Palmitic acid	Antipsychotic medication, Treatment of schizophrenia, Anti Inflammatory property, Anti oxidants, Anti-Atheros clerotic
2.	4H-1-Benzopyran-4-one[or]Chromanone	Antimicrobial activity, Anti oxidants, Anti-inflammatory, Anti-cancer
3.	Phytol	Anxiolytic, antidepressant, anticonvulsant, hypolipodemic and antischistosomal properties
4.	Octadecanoic acid [or] Stearic acid	Emulsifying agent, Solubilizing agent, tablet and capsule lubricant, Lotions, Prevent oxidation, Medicine manufacturing,
5.	Oleic acid	Pharmaceutical solvent, asthma inhalers
6.	Eicosanoic acid	It is used in the manufacture of pharmaceuticals
7.	Flavone	Anti-inflammatory, anti-allergenic, anti- viral, anti- cancer, anti-asthma effects, anti-proliferative anti-invasive, anti-metastatic and anti oxidant activities, Reduce the blood pressure and plasma glucose level.
8.	Docosanoic acid [or] Behenic acid	Docosanoic acid significantly raises serum total and low- density lipoprotein cholesterol concentrations.
9.	Docosanoic acid,3,4,-dimethyl ester.	For treating wounds, Ulcers, burns, Scars, keloids, Antipyretic or anti-inflammatory agents.

Table 4: Antibacterial activity of Annapathi chenduram.

Organisms	Zone of inhibition (mm)											
	<i>Bacillus subtilis</i>			<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Klebsiella pneumoniae</i>		
Concentration (µg/ml)	25 µg	50µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg	25 µg	50 µg	100 µg
A.Chenduram	7	13	19	-	7	15	-	7	10	8	10	14
Streptomycin (20µg/ml)	24			26			18			22		

4. CONCLUSIONS

The Anapathi chenduram, known as medicinal are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, related active metabolites. It has been examined meticulously for its phytochemical and pharmacological activities. From the aforementioned explanation, it is noticeable that has been used as an important extracts exhibited an antibacterial activity. Further study is required to identify the active compounds, toxicity and safety of these Anapathi chenduram and eventually clinical evaluations.

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REFERENCES

- Retnam KR and De Britto AJ. Phytochemical analysis of a medicinal plant Hybanthus enneaspermus (L) F. Muell. J. Econ. Taxon. Bot., 2003; 27(3): 701-706.
- Balandrin MJ and Klocke JA. Medicinal, aromatic and industrial materials from plants, In Y.P.S. Bajaj (ed) Biotechnology in agriculture and Forestry. Medicinal and Aromatic plant, Springer-Verlag, Berlin Heidelberg, 1988; 4: 1-36.
- Bhandary SK, Kumari SN, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *punica granatum* peel, whole fruit and seeds. Nitte University Journal of Health Science, 2102; 2(4).
- Prasad NR. Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. Journal of Medicinal Plants Research, 2008; 2: 268-270.
- Nithya TG, Sivakumar S, Sangeetha K, Antibacterial activity of Vallarai Chooranam against Human Pathogens, International J. of Pharm Tech Research, 2012; 4(1): 162-168.
- Shree Devi MS, Ravichandiran V and Jayakumari S. Phytochemical Study on *Solanum Xanthocarpum* Chooranam, Int. J. of Frontiers in Science and Technology, 2014; 2: 2.
- Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C and Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. John Wiley & Sons A/S., 2012; 1-2.
- Yoshikoshi M, Yoshiki Y, Okubo K, Seto J, Sasaki Y. Prevention of Hydrogen Peroxide Damage by Soybean Saponins to Mouse Fibroblasts. Planta Med., 1996; 62(3): 252-255.
- Bhandary SK, Suchetha KN, Vadisha SB, Sharmila KP, Mahesh PB. Preliminary phytoscreening of various extracts of *Punica Grantanum* Peel, whole fruits and seeds, Nitte University Journal of Health Science, 2012; 2(4): 34-38.
- Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother, 2001; 48.
- Alisi CS, Ojiako OA, Osuagwu CG, Onyeze GOC. Free radical scavenging and *in-vitro* antioxidant effects of ethanol extract of the medicinal herb *Chromolaena odorata* Linn. British Journal of Pharmaceutical Research, 2011; 1(4): 141-155.
- Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. Journal of Agricultural and Food Chemistry, 2005; 53: 1408-1416.
- Manashi B, Milness M, Williams C, Balmoori J, Ye X, Stohs S, Bagchi D. Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract. Nutrition Research, 1999; 19.
- Kumar P, Kumaravel PS, Lalitha C., Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*, African Journal of Biochemistry Research, 2010; 4(7): 191-195.
- CLSI-Clinical and laboratory standards institute. Performance standards for antimicrobial disk susceptibility tests, 10th ed. Approved standard, Document M02-A10 2009; CLSI, Wayne, PA.
- WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO Library Cataloguing-in-Publication Data; Spain, 2007.
- Harborne JB, Phytochemical methods: A guide to modern technique of plant analysis, Champman and Hall, London, 1998.
- Shrivastav S, Rakesh K, Kumar SS, Kumar P. Anti-Psoriatic and phytochemical evaluation of *Thespesia populne a* barks extracts. International Journal of Pharmacy and pharmaceutical Sciences, 2009; 1: 176-185.