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Comparative Study Of Secondary Metabolites In *Annona Reticulata* With Its Antibacterial Activity In Various Extracts

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ABSTRACT

Phytochemical analysis involves the determination of the presence of phytochemical constituents in the crude samples of the medicinal plants. Phytochemical tests are carried out first to establish the presence or otherwise of some specific phytochemicals. The plant also found to be rich in minerals such as Ca, P, K, Mg, Na, Cl, S Mn, Zn, Fe, Cu, Se, Co, Ni and Cr. The agar well diffusion method was used to determine the antibacterial activity using Bauer-Kirby method (Bauer et al., 1966). The different extracts from different plant parts of *A. reticulata* were screened for antibacterial activity against five clinically important bacteria. These bacteria were procured from National Collection for Industrial Microorganism (NCIM), India and American Type Culture Collection (ATCC), USA Among test bacteria three Gram negative bacteria i.e. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were used while among Gram positive bacteria *Staphylococcus aurous* and *Streptococcus pneumonia* were used. The pure cultures of the bacteria were maintained during the study in nutrient agar media (HI Media, India).

Keywords: Annonaceae, Phytochemical constituents, Phytochemical screening.

INTRODUCTION

Natural products from plants, trees, shrubs and other microorganisms represent an ever-ending field of fascination. Use of natural products in all fields has been a regular phenomenon in the past, especially for the treatment of various ailments. With the advent of synthetic organic chemistry, the pace of natural product chemistry slowed down owing to various reasons. But, again with the knowledge of multi drug resistance and other side effects caused by the synthetic compounds, natural product chemistry resurged. Another important reason for the resurgence of the use of natural products in the pharmaceutical field is because certain drugs cannot yet be synthesized economically and are obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds (Rates, 2001). Considering on the aforesaid facts, the present work has been undertaken with a view to highlight the importance of natural products.

The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The main advantages claimed for therapeutic uses of medicinal plants in diverse ailments are their safety in addition being economical, efficient and their effortless availability. Because of these advantages the medicinal plants have been extensively used by the traditional medical practitioners in their daily practice.

According to the WHO survey 80% populations living in the developing countries really almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants & pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us a number of very significant lifesaving drugs used in the modern medicine. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically (Balsndrin et al., 1985; Cragg et al., 1997).

A.reticulata is a small deciduous or semi-evergreen tree listed as an "agricultural weed, environmental weed, garden thug, naturalized, weed" in the Global Compendium of Weeds (Randall, 2012) and is considered an invasive species by CeNBIO. The genus Annona, commonly known as the custard-apple genus, consists of some 125 species with some species widely cultivated for their edible fruits and often becoming naturalized beyond their native range of tropical America and Africa (Wagner et al., 2014). The name of the genus 'Annona' is derived from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the variety of species in this genus. The species *A. reticulata* shared the common name 'custard apple' and 'cherimoya' and 'atimoya'

MATERIAL AND METHOD

Collection of Sample

The whole plant of *Annona reticulata* was collected from Jawaharlal Nehru Krishi Vishwavidyalaya of Jabalpur region of central India. The plants were uprooted and immediately transported to the laboratory. After washing with running tap water for 1 h, leaves, flowers, stem and seeds of *Annona reticulata* were separated and dried under shade. Taxonomic identification was carried out using available literature. The different plant parts of A. *reticulata* were ground and the powder obtained was sieved through a 100 micro gram test sieve.

Study of Phytochemicals

For the Screening, the phytochemicals were extracted from *A. reticulata* powder sequentially with water, methanol, ethyl acetate and petroleum ether.

For this, 10 g of dried plant powder was extracted with water using cold percolation method. The extract was concentrated under vacuum up to 20 ml and kept in refrigerated until use. The residue after cold percolation was dried and used for further extraction sequentially with methanol, ethyl acetate and petroleum ether using a Soxhlet extractor. The extracts were concentrated to 20 ml as describe above. Qualitative tests for various secondary metabolites were performed using methods described by Trease and Evans and Harborne.

Antibacterial activity by agar well-diffusion

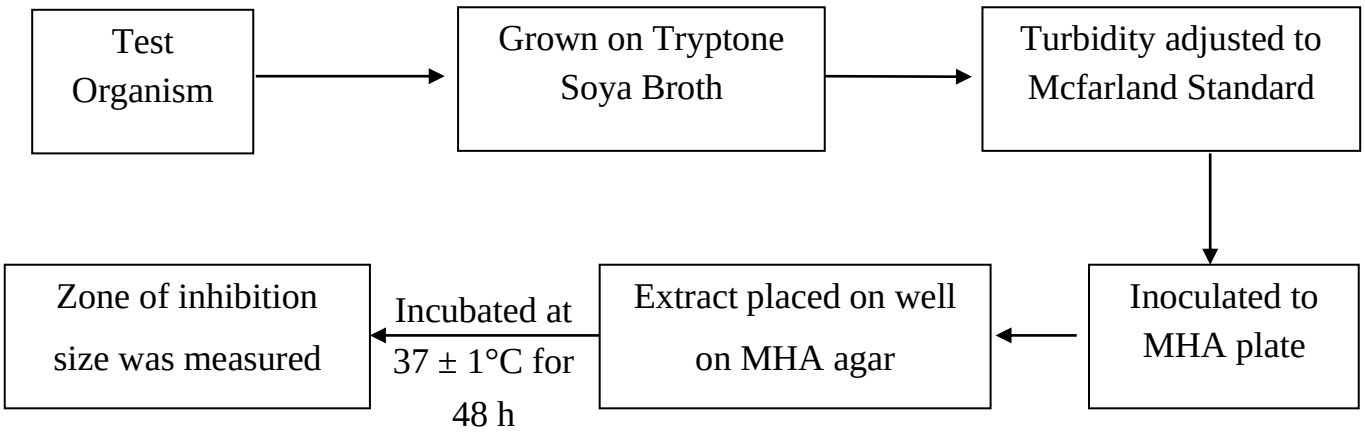

Figure 1.1 Flow sheet of antimicrobial susceptibility testing by well diffusion

The agar well diffusion method was used to determine the antibacterial activity using Bauer-Kirby method (Bauer et al., 1966). The different extracts from different plant parts of *A. reticulata* were screened for antibacterial activity against five clinically important bacteria. These bacteria were procured from National Collection for Industrial Microorganism (NCIM), India and American Type Culture Collection (ATCC), USA. Among test bacteria three Gram negative bacteria i.e. *Escherichia coli* NCIM 2256, *Klebsiella pneumoniae* NCIM 5432 and *Pseudomonas aeruginosa* NCIM 2200 were used while among Gram positive bacteria *Staphylococcus aureus* NCIM 2079 and *Streptococcus pneumoniae* ATCC 49619 were used. The pure cultures of the bacteria were maintained during the study in nutrient agar media

Antifungal screening by well-diffusion

The antifungal screening was carried by well diffusion method, similar to antibacterial screening. The different extracts from different plant parts of *A. reticulata* were screened for antifungal activity against *Candida albicans* ATCC 10231 procured from American Type Culture Collection (ATCC), USA. Miconazole (30 µg) acted as control antifungal agent.

RESULTS

1.Phytochemical Screening

Qualitative phytochemical analysis confirmed the presence of several secondary metabolites across the extracts obtained from different plant parts. Alkaloids were detected in methanol and aqueous extracts of the stem, as evidenced by positive Mayer's and Wagner's tests.
Saponins were exclusively present in the aqueous extract.

- Flavonoids identified in both aqueous and methanol extract.
- Tannins and triterpenes were observed in the methanol extract only.
- Sterols were confirmed through a positive Salkowski test in methanol extract.
- Cardiac glycosides were present solely in the petroleum ether extract.
- Anthraquinone and resins were absent in all samples.

Quantitative estimation further revealed that seeds contained the highest alkaloid content (mean 5.481mg/g), while Saponins content peaked in leaves (0.882 mg/g). Terpenoids were most abundant in leaves and fruits.

2. Antibacterial activity

The antibacterial efficacy varied across extracts and plant parts.

Seed extracts: Methanol and ethyl acetate extracts demonstrated inhibition against *Escherichia coli* and streptococcus pneumoniae (Zones:10-15mm), while methanolic extract showed moderate inhibition against *Pseudomonas aeruginosa*. Aqueous and petroleum ether extracts exhibited no activity.

Stem extracts: Methanol extract Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae (zones: 8-12 mm). Ethyl acetate extract showed weak inhibition of *S. aureus*. No inhibition was recorded for aqueous or petroleum ether extracts.

Leaf extracts: Only the methanolic extract displayed antibacterial activity with inhibition zones of 10 mm against *S.aureus*, *K. pneumonia* and *P.aeruginosa*.

Fruit extracts: The methanolic extract demonstrated significant inhibition against *S.aureus* (22 mm) and moderate activity against *P.aeruginosa* (12 mm).Other solvents showed no antibacterial effects.

Table-1: Total Alkaloids content (mg equivalent per grams) in different parts of *Annona reticulata* plant. Values are presented as mean ±SD, n=3.

S. No.	Plant part	Replicate			Mean	SD ±
		1	2	3		
1.	**Fruit**	1.584	1.617	1.531	**1.577**	0.043
2.	**Stem**	0.256	0.311	0.279	**0.282**	0.028
3.	**Seed**	5.484	5.412	5.547	**5.481**	0.068
4.	**Leaf**	4.681	4.710	4.610	**4.667**	0.051

Table-2 : Total Saponins content (mg equivalent per grams) in different parts of *Annona reticulata* plant. Values are presented as mean ±SD, n=3.

S. No.	Plant part	Replicate			Mean	SD ±
		1	2	3		
1.	**Fruit**	0.152	0.167	0.173	**0.164**	0.011
2.	**Stem**	0.352	0.311	0.279	**0.314**	0.037
3.	**Seed**	0.398	0.369	0.395	**0.387**	0.016
4.	**Leaf**	0.882	0.894	0.869	**0.882**	0.013

Table–3 : Total Terpenoids content (mg equivalent per grams) in different parts of *Annona reticulata* plant Values are presented as mean ±SD, n=3.

S. No.	Plant part	Replicate			Mean	SD ±
		1	2	3		
1.	**Fruit**	0.367	0.355	0.369	**0.364**	0.008
2.	**Stem**	0.168	0.311	0.279	**0.253**	0.075
3.	**Seed**	0.236	0.287	0.248	**0.257**	0.027
4.	**Leaf**	0.364	0.397	0.359	**0.373**	0.021

Table - 4: Antibacterial activity of *A. reticulata* seed

Test Bacteria	Diameter of inhibitory zone (mm)			
	Aqueous	**Methanol**	**Ethyl Acetate**	**Petroleum Ether**
Escherichia coli NCIM 2256	+	10	11	-
Klebsiella pneumonia NCIM 5432	-	-	-	-
Pseudomonas aeruginosa NCIM 2200	-	12	15	-
Staphylococcus aureus NCIM 2079	-	22	-	-
Streptococcus pneumonia ATCC 49619	-	10	10	-

Table 5: Antibacterial activity of *A. reticulata* stem

Test Bacteria	Diameter of inhibitory zone (mm)			
	Aqueous	**Methanol**	**Ethyl Acetate**	**Petroleum Ether**
Escherichia coli NCIM 2256	-	12	-	-
Klebsiella pneumonia NCIM 5432	-	8	-	-
Pseudomonas aeruginosa NCIM 2200	-	-	-	-
Staphylococcus aureus NCIM 2079	-	10	8	-
Streptococcus pneumonia ATCC 49619	-	-	-	-

Table-6: Antibacterial activity of *A. reticulata* leaf

Test Bacteria	Diameter of inhibitory zone (mm)			
	Aqueous	**Methanol**	**Ethyl Acetate**	**Petroleum Ether**
Escherichia coli NCIM 2256	-	-	-	-
Klebsiella pneumonia NCIM 5432	-	10	-	-
Pseudomonas aeruginosa NCIM 2200	-	10	-	-
Staphylococcus aureus NCIM 2079	-	10	-	-
Streptococcus pneumonia ATCC 49619	-	-	-	-

Table - 7: Antibacterial activity of *A. reticulata* fruit

Test Bacteria	Diameter of inhibitory zone (mm)			
	Aqueous	**Methanol**	**Ethyl Acetate**	**Petroleum Ether**
Escherichia coli NCIM 2256	-	-	-	-
Klebsiella pneumonia NCIM 5432	-	-	-	-
Pseudomonas aeruginosa NCIM 2200	-	12	-	-
Staphylococcus aureus NCIM 2079	-	22	-	-
Streptococcus pneumonia ATCC 49619	-	-	-	-

DISSCUSSION

The finding highlights that *Annona reticulata* is a promising source of bioactive phytochemicals with measurable antibacterial potential. Notably, methanolic extracts consistently demonstrated higher antibacterial activity compared to other solvents. This can be attributed to the higher solubility of active compounds like alkaloids, flavonoids and triterpenoids in polar solvents such as methanol.

The results corroborate earlier studies indicating the rich phytochemical composition and broad-spectrum antimicrobial effects of Annona reticulata (Gupta et al., 2014; Nawab et al; 2011). The selective absence of antibacterial activity in aqueous and petroleum ether extracts reinforces the importance of solvent selection in phytochemical investigations(Balakrishna & Kuppusamy, 2013).

Furthermore, the pronounced activity against staphylococcus aureus suggests that methanolic extracts may be particularly effective against Gram-positive organisms were more susceptible to plant-derived secondary metabolites (Saha &Verma, 2016).

CONCLUSION

This study demonstrates that different parts of parts of *Annona reticulata* contain significant phytochemicals particularly alkaloids, flavonoids, Saponins and triterpenoids are likely responsible for the observed antibacterial effects. Among the extracts tested, methanol extract showed the most pronounced activity especially against staphylococcus aureus and pseudomonas aeruginosa.

These results validate the traditional uses of *Annona reticulata* and highlights is potential as a source of natural antimicrobial agents. Future research should focus on isolating and characterizing individual active compounds and evaluating their efficacy through in vivo studies.

REFERENCE

Balsndrin MF, Klocke JA, Wretele ES, Boilinger WH. Content and purity of extractsolasodine in some available species of solanum. Sciences and culture.1985; 56(5): 214-216.

Randall RP. A Global Compendium of Weeds. Perth, Australia: Department of Agriculture and Food Western Australia, 2012; 1124.

Rates SMK. Plants as source of drugs. Toxicon. 2001; 39: 603–613.

Wagner WL; Herbst DR; Lorence DH, 2014. Flora of the Hawaiian Islands website. Washington DC, USA: Smithsonian Institution.

Zaman K., Pathak K. Pharmacognostical and phytochemical studies on the leaf and stem bark of *Annona Reticulata* Linn. J Pharmacogn Phytochem. 2013; 1:1–8.