

Phytochemical Screening of Leaves of *Averrhoa carambola* L. In Different Solvents

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Abstract: Plants acquire bioactive complexes have been the centre of attention of recently research owing to their health encouraging effects. *Averrhoa carambola* L. plant belongs to Oxalidaceae family. The current examination was carried out to estimate the qualitative phytochemical analysis of leaves of *Averrhoa carambola* L. Here, two solvents were used for the phytochemical screening named Methanol and Hexane. Two different solvents screening showed the presence of Alkaloids, Phenols, Saponins, Terpenoids, Flavonoids. Since the plant suppress high quantities of these new bio-active probable compounds, it is well founded to have huge number of pharmacological activities such as Antibacterial, Antioxidant, Antifungal, Antiulcer, Anti-inflammatory, diuretics activities and are being utilized for the therapy of different disease or disorder in the original system of medicine.

Key Words: *Averrhoa carambola* L., Phytochemicals, Secondary metabolites.

1. INTRODUCTION:

The medicinal plants are effective for recovering as well as for curing of human disorders due to the presence of phytochemicals compounds. Phytochemicals are naturally taken out in the medicinal plant's roots, leaves, stem bark and fruits that have protection mechanism and defend from different diseases. Natural outcome from plants called as secondary metabolites are the final products of primary metabolites such as amino acid, lipid, carbohydrate, chlorophyll etc. They are blend large diversity of chemical compounds known as secondary metabolites which involve Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Steroids, Phenols etc. *Averrhoa carambola* L. is famous medicinal plant but it also having various phytochemicals like Alkaloids, Flavonoids, Phenols, Saponins, Terpenoids. The study was focused on the screening of leaves of *Averrhoa carambola* L. in methanolic and Hexane solvents.

2. MATERIAL AND METHODOLOGY

2.1 Collection of Plant material

The Leaves of *Averrhoa carambola* plant was collected from Navsari Agricultural University, Navsari district, Gujarat in month of January 2022. The plant was recognized by Prof. Bharat Maitreya, Department of Botany, Bioinformatics, Climate change and Impacts Management, Gujarat University, Gujarat. The plant part selected was only leaves.

2.2 Preparation of Plant extract:

10 gm powder of dry leaves of *Averrhoa carambola* was dissolved in 150 ml of solvent (Methanol, Hexane) in a conical flask. The top portion of conical flasks were enveloped with aluminium foil paper with the help of rubber bands. After then conical flasks were kept on the shaker for 24 hours. Therefore, all the components were dissolved perfectly. After 24 hours, the extracts were strained using the Whatman paper No.1 into the petri dishes. Before the filtration, the petri dishes were weighed to know its weight. After filtration the extracts were remain to vaporize properly. Furthermore, petri dishes were weighed again to know their weight after filtration. then petri dishes were stored in a dry and cool place.

3. QUALITATIVE ANALYSIS OF SECONDARY METABOLITES:

The extract was tested for the presence of bioactive compounds by using following standard methods.

Test For Alkaloids

- Mayer's Test: 1ml filtrate was treated with 2ml Mayer's reagent – cream colour precipitation indicates the presence of alkaloids.
- Dragendroff's Test: 1ml filtrate was treated with 2ml Dragendroff's reagent – orange red colour precipitation indicates the presence of alkaloids.
- Wagner's Test: 1ml filtrate was treated with 2ml Wagner's reagent- reddish brown colour indicates the presence of alkaloids.

Test For Flavonoids

- Zinc hydrochloride's Test: 1ml extract was treated with zinc dust and conc. HCl-Formation of red colour indicates the presence of flavonoids.
- Pew Test: 1ml of extract was treated with pieces of metallic magnesium and 2-3 drops conc. HCl was added – formation of brownish colour indicates the presence of flavonoids.
- H₂SO₄ Test: 1ml extract was treated with few drops of H₂SO₄ – orange colour precipitation indicates the presence of flavonoids.
- Lead acetate Test: 1ml extract was treated with a few drops of 10% lead acetate solution- yellow precipitate indicates the presence of flavonoids.

Test for Phenols

- Ferric chloride Test: Few ml extract was treated with 5ml D.W. add few drops of 5% ferric chloride and boil – Blue green colour indicates the presence of Phenols.
- Lead acetate Test: Few ml extract was treated with 3ml 10% lead acetate solution – Bulky white precipitate indicates the presence of Phenols.
- Potassium dichromate Test: Few ml extract was treated with Potassium dichromate solution – orange colour formation indicates the presence of the phenols.
- Folin ciocalteu Test: 1ml extract was taken. Added 1ml Folin ciocalteu reagent – Blue green colour indicates the presence of phenols.

Test For Tannins

- Ferric chloride Test: small quantity of extract was mixed with water and heated in water bath, the mixture was filtered and 0.1% ferric chloride solution was added- Blue/dark green colour indicates the presence of Tannins.
- Lead acetate Test: few ml extract treated with 1ml lead acetate solution – White precipitation shows the presence of Tannins.
- Potassium dichromate Test: Few ml extract was treated with Potassium dichromate solution – orange colour formation indicates the presence of the Tannins.

Test For Saponins

- Frothing Test: About 0.5mg of extract added 3 drops olive oil was shaken with 5ml of D.W.- small bubbles of froth indicates the presence of saponins.
- Foam Test: 1ml extract taken. Added 20ml D.W. and shaken vigorously- formation of foam indicates the presence of saponins.

Test For Steroids

- Liebermann Burchard Test: Take Few ml extract was treated with few drops acetic anhydride then boil. Cool it. Add conc. sulphuric acid. – Brown ring formation indicates the presence of Steroids.
- Liebermann Sterol Test: Take a Few ml extract then add 1 ml Glacial acetic acid add 1 drop conc. H₂SO₄ – play of colours from red, violet, blue to green indicates the presence of Steroids.
- Salkowski's Test: Take 2 ml extract is Shaked with 1 ml chloroform then add conc. H₂SO₄ was added side by side. – Red colour indicates the presence of Steroids.

Test for Glycosides

- Ferric chloride Test: Take 1 ml extract then treated with 1 ml glacial acetic acid and 1 ml 2% FeCl₃ solution add 1 ml of Conc. H₂SO₄- Formation of 2 layers. Upper layer is brown and lower layer is blue indicates the presence of glycosides.
- Bromine H₂O Test: Take 1 ml extract was dissolved in few ml bromine H₂O-Yellow colour precipitation indicates the presence of glycosides.
- Ammonia Test: 1 ml filtrate was treated with 3 ml chloroform, then shake it well and add 10% ammonia solution – Pink colour formation indicates the presence of glycosides.

Test for Carbohydrate/Sugar

- Fehling Test:** Take 1 ml extract was treated with 1 ml Fehling A and 1 ml Fehling B reagent then boil for 2 minutes- Brick red colour precipitation indicates the presence of carbohydrate.
- Benedict Test:** Take 1 ml extract then add 1 ml Benedict reagent – Reddish brown precipitation indicates the presence of carbohydrate.
- Iodine Test:** Take 1 ml extract add 3 drops of iodine solution – Blue colour precipitation indicates the presence of carbohydrate.

Test for Protein/Amino acid

- Millon's Test:** Take 1 ml extract add 1 ml Millon's reagent – White colour precipitation indicates the presence of protein.
- Ninhydrin Test:** Take 2 ml extract add 2 drops Ninhydrin solution- purple colour precipitation indicates the presence of protein.

Test for Terpenoids

- Salkowski Test:** Few ml of extract was mixed with 2 ml of chloroform and 3 ml of Conc.H₂SO₄ was carefully add to form layer- reddish brown colour ring formation indicates the presence of terpenoids.
- Chloroform Test:** Take 1 ml extract add 2 ml chloroform then add 3 ml conc.H₂SO₄ it forms a layer- red brown colour indicates the presence of terpenoids.
- Copper acetate Test:** 1 ml extract was treated with 1-2 drops of copper acetate solution- emerald green precipitation indicates the presence of terpenoids.

Test for Fixed oil/Fats

- Oil Stain Check:** extract was poured dropwise on a filter paper – if oil stain is observed then it indicates the presence of fats.

4. RESULT AND DISCUSSION:

The Qualitative analysis of methanolic leaves extract shows the presence of secondary metabolites such as Alkaloids, Flavonoids, Phenols, Tannins, Saponins and Terpenoids. The Qualitative analysis of hexane leaves extract shows the presence of secondary metabolites such as Alkaloids, Phenols and Saponins.

Sr. No.	Phytochemicals	Test	Results (ME)	Results (HE)
1	Alkaloids			
		Mayer's Test	+	+
		Dragendroff's Test	+	+
		Wagner's Test	+	+
2	Flavonoids			
		Zinc hydrochloride's Test	+	-
		Pew Test	+	-
		H ₂ SO ₄ Test	+	-
		Lead acetate Test	+	-
3	Phenols			
		Ferric chloride Test	-	-
		Lead acetate Test	+	-
		Potassium dichromate Test	+	+
		Folin ciocalteu Test	+	+
4	Tannins			
		Ferric chloride Test	-	-
		Lead acetate Test	+	-
		Potassium dichromate Test	+	-
5	Saponins			
		Frothing Test	+	+

		Foam Test	+	+
6	Steroids			
		Liebermann Burchard Test	-	-
		Liebermann sterol Test	-	-
		Salkowski's Test	-	-
7	Glycosides			
		Ferric chloride Test	-	-
		Bromine H ₂ O Test	-	-
		Ammonia Test	-	-
8	Carbohydrate/Sugar			
		Fehling Test	-	-
		Benedict Test	-	-
		Iodine Test	-	-
9	Protein/Amino acid			
		Millons Test	-	-
		Ninhydrin Test	-	-
10	Terpenoids			
		Salkowski's Test	+	-
		Chloroform Test	+	-
		Copper acetate Test	+	-
11	Fixed Oil or Fats			
		Oil stain check	-	-

Table-1 Showing Qualitative analysis of secondary metabolites in methanolic, hexane extract of leaves
 Where + indicates Presence and – indicates Absence

5. CONCLUSION:

Averrhoa carambola L. is a medicinal plant. It has different phytochemicals like Alkaloids, Phenols, Saponins, Terpenoids, Flavonoids etc. Due to the presence of secondary metabolites, it has different pharmacological activities such as antimicrobial, anti-inflammatory, antiulcer, antibacterial, antifungal, antitumor, antidiabetic etc.

REFERENCES:

1. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A and Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*. 2014; 5(2):95-100
2. Harborne, J.B. 1973. *Phytochemicals Methods*. Chapman and Hall Ltd., London, pp. 49-188.
3. Kumar R, Sharma S, Devi L. Investigation of Total Phenolic, Flavonoid Contents and Antioxidant Activity from Extract of *Azadirachta indica* of Bundelkhand Region. *International Journal of Life Sciences and Scientific Reserch*. 2018, 4(4):1925-1933.
4. Manda H, Vyas K, Pandya A, Singhal G., A complete review on: *Averrhoa carambola*., *World J Pharm Pharm Sci.*, 2012; 1(1): 17- 33.
5. Nanna RS, Banala M, Pamulaparathi A, Kurra A, Kagithoju S. Evaluation of Phytochemicals and Fluorescent Analysis of Seed and Leaf Extracts of *Cajanus cajan* L. *International Journal of Pharmaceutical Sciences Review and Research*. 2013; 22(1):11-18.
6. Pandey A and Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*. 2014; 2(5):115-119.
7. Pant DR, Pant ND, Saru DB, Yadav UN, Khanal DP. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. *J Intercult Ethnopharmacol*. 2017; 6(2):170-176.
8. Raaman N. *Phytochemical Techniques*. New India Publishing Agency, New Delhi, 2006, 19-24.

9. Ray S, Chatterjee S, Chakrabarti CS. Antiproliferative Activity of Allelo chemicals Present in Aqueous Extract of *Synedrella nodiflora* (L.) Gaertn. In Apical Meristems and Wistar Rat Bone Marrow Cells. *Iosr Journal of Pharmacy*. 2013; 3(2):1-10.
10. Shaikh, J.R., & M.K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608.
11. Sheel R, Nisha K, Kumar J. Preliminary Phytochemical Screening of Methanolic Extract of *Clerodendron infortunatum*. *IOSR Journal of Applied Chemistry*. 2014; 7(1):10-13.
12. Silva GO, Abeysundara AT, Aponso MM. Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*. 2017; 5(2):29-32
13. Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region. *International Journal of Life Sciences Scientific Research*. 2017; 3(6):1451-1458.
14. Sofowra, A. 1993. Medicinal Plants and Traditional Medicine In Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
15. Sona Rajashree, B., & Sangeetha, V. S. PHYTOCHEMICAL SCREENING AND IN VITRO ANTIDIABETIC ACTIVITY OF *AVERRHOA CARAMBOLA* LINN. LEAF EXTRACTS.
16. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*. 2011; 1(1):98- 106.
17. Trease, G.E., Evans, W.C. 1989. Pharmacognosy, 11th edn., Bailliere Tindall, London, pp. 45-50.
18. Tyagi T. Phytochemical Screening of Active Metabolites Present in *Eichhornia Crassipes* (Mart.) Solms and *Pistia stratiotes* (L.): Role in Ethanomedicine. *Asian Journal of Pharmaceutical Education and Research*. 2017; 6(4):40-56.
19. Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Olea dioica* Roxb., infected with the rust fungus *Zaghouania oleae* (E.J. Butler) Cummins and noninfected plants. *Journal of Pharmacognosy and Phytochemistry*. 2014; 3(4):69-72.
20. Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, 3(12).