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RESEARCH ARTICLE

STUDY OF QUALITATIVE PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF LAWSONIA INERMIS L. AND JATROPHA GOSSYPIFOLIA L

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Abstract

The present paper reports the Qualitative phytochemical analysis of leaf extracts of *Lawsoniainermis* L. (Lythreaceae) and *Jatropha gossypifolia* L. (Euphorbiaceae) which are used by the tribal and rural people for curing different ailments in the West Singhbhum district of Jharkhand, India. Phytochemical tests confirmed the presence of primary and secondary classes of Phytochemicals in the ethanolic, methanolic and aqueous leaf extracts of *L. inermis* L. and *J. gossypifolia* L. such as Carbohydrates, proteins, Phenols, Tannin, Flavonoids, Saponins, Glycosides, Steroids, Terpenoids.

Introduction:-

Medicinal plants are one of the best natural gifts that are used to cure several diseases and disorders of humans and animals. About 70% of the medicinal plants came to the attention of the pharmaceutical industry, because of their use in traditional medicines (Abelson, 1990). India is one of the pioneers in the development and well-documented Indigenous systems in Ayurveda, Siddha, and Unani. Kosalage and Fursule (2009) reported that WHO's latest estimation is about 70%-95% population of developing countries and 70%-80% population of developed countries used complementary and alternative medicine for their primary healthcare.

Traditional medicines are becoming popular due to the high toxicity and adverse effects of orthodox medicament. Secondary metabolites are biologically active compounds frequently present in small quantities as compared to primary metabolites (Farnsworth, 1977). Although historically plants have provided a good source of antibiotic agents in the fight against microbial infections, by the advent of antibiotics in the 1950's the use of plant derivatives as antimicrobials has been ignored (Cowan,1999; Iwu,1999). Tudu and Sinha (2015 &2017) reported the medicinal plants of the east and west singhbhum district of Jharkhand, India.

Jatropha gossypifolia L. is used as a therapeutic agent in different ways. The decoction of leaves is used for bathing wounds (Edeoga*et.al*., 2015). In Latin America and the Caribbean, people used leaf baths for sores, sprains, rashes, and bewitchment (Tanaka *et.al*., 2002). Crushed leaves mixed with breast milk are applied on the head to cure infantile diarrhea (Dhale*et.al*., 2010). A decoction of leaves is used for stomach aches and venereal disease, and as a blood purifier (Kirtikar and Basu, 1996). The extracts of fresh leaf applied with crushed leaf are used by herbal practitioners and local people to stop bleeding from the skin and nose (Saini *et.al*., 2015).

Lawsoniainermis L. (Lythraceae) is used as antiparasitic, antimicrobial, antimycotic, and virucidal activity due to the presence of its major bioactive constituents like mannite, tannic acid, mucilage, gallic acid, and 2-hydroxynaptoquinone (lawsone) (Muhammad and Muhammad,2005). In folk medicines, henna (*Lawsoniainermis*L.)

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has been used as an astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative, and also as therapeutic against amoebiasis, headache, jaundice, and leprosy (Raja Sekaran, 2001).

The Phytochemical studies revealed the presence of a variety of compounds like Carbohydrate, Protein, Phenol and Tannin, Flavonoids, Saponins, Steroids, Glycosides, Alkaloids, Terpenoids, and Quinine (Sofowora,1993; Ram and Sinha,2017).

Materials and Methods:-

Preparation of Plant Extracts

Fresh and mature leaves of *Jatropha gossypifolia* L. and *Lawsoniainermis* L. were collected fromthe campus of Kolhan University, Chaibasa, Jharkhand, India in August 2022. The leaves were washed with distilled water and kept for drying in the shade for 7-10 days. The dried leaves were ground into a powder with the help of a mixer grinder and kept in an air-tight container.

10 g of the leaf powder was soaked in 100 ml of ethanol, methanol, and Double Distilled water separately in a conical flask (Gaikwad, 2012) and kept on a magnetic stirrer for 30 mins at room temperature. After 72 hours, it was again kept on a magnetic stirrer for 30 minutes and then filtered with the help of Whatman filter paper 1 (125mm Cat no 1001 125). The filtrate was stored in an air-tight container in the refrigerator for future use.

Test for Primary Phytochemicals

Test for Carbohydrate

Three methods were used for the detection of carbohydrates.

A1] Fehling's test method:

Equal volumes ofFehling A and Fehling B reagents were mixed with 2ml of crude leaf extract and boiled gently. The appearance of a brick-red precipitate at the bottom of the test tube confirmed the presence of reducing sugar.

A2] Benedict's test method:

2 ml of leaf extract was added with 2 ml of Benedict's reagent and then boiled, the formation of a reddish-brown precipitate indicated the presence of the carbohydrate.

A3] Iodine test method:

A few drops of iodine solution were added to 2 ml of leaf extract, the appearance of a dark blue or purple color indicated the presence of carbohydrates.

B] Test for Protein

Two methods were used for the detection of Protein.

B1] Millon's test method:

2 ml of Millon's reagent was added to 2 ml of the leaf extract, appearance of a white precipitate which turned into red on gentle heating confirmed the presence of protein.

B2] Ninhydrin test method:

2 ml of 0.2% solution of Ninhydrin was added in 2 ml of leaf extract and then boiled for 3-5 min, appearance of violet color indicated the presence of proteins.

Test for Secondary Phytochemicals

C] Test for Flavonoids

Two methods were used for the detection of the flavonoids:

C1]Shinoda Test Method:

In this test few fragments of magnesium ribbons were added to 2 ml of leaf extracts and then gradually dropwise concentrated HCl was added to the extracts. The appearance of pink scarlet color after a few minutes confirmed the presence of flavonoids.

C2]Alkaline Reagent Test Method:
2 ml of 2% solution of NaOH was added to 2 ml of leaf extract, and an intense yellow color was formed which turned colorless on the addition of a few drops of diluted sulphuric acid indicating the presence of Flavonoids.

D] Test for Steroids
Two methods were used for the detection of Steroids

D1] Chloroform Test Method:
2 ml of plant extracts were added in 2 ml of chloroform, and then 2 ml of Concentrated Sulphuric Acid (H_2SO_4) was added sidewise. The appearance of red color in the lower chloroform layer indicated the presence of steroids.

D2] Acetic Acid Method:
2 ml ofAcetic Acid was added to 2 ml of the plant extracts, then 1 ml of concentrated H_2SO_4 was added dropwise. The presence of blue-green color confirmed the presence of Steroids.

E] Test for Alkaloids
E1] Mayer's and Wagner's reagents Test Method:
2 ml of1% Hydrochloric Acid was added to 2 ml of the plant extracts and then heated gently. The Mayer's and Wagner's reagents were added in equal proportion. Turbidity of the resulting precipitate indicated the presence of Alkaloids.

F] Test for Terpenoids
F1] Chloroform Test Method:
2 ml of the plant extracts were dissolved in Chloroform and left for evaporation to dry and then 2 ml of concentrated sulphuric acid was added sidewise and heated for a few mins. The appearance of a greyish color indicated the presence of Terpenoids.

G] Test for Glycosides
G1] Keller-Kilani Test Method:
2 ml of glacial acetic acid was added to 2 ml of the plant extract and followed by 1-2 drops of 2% solution of Ferric Chloride ($FeCl_3$). The mixture was poured into another test tube which contained 2 ml of concentrated Sulphuric acid(H_2SO_4). The appearance of a brown ring at the inner phase indicated the presence of cardiac glycosides.

H] Test for Saponin
H1] Foam Test Method:
5 ml of distilled water was added to 2 ml of leaf extracts and then it was shaken vigorously. The formation of stable foam indicated the presence of Saponins.

I] Test for Phenols and Tannin
I1] Ferric Chloride Test Method:
2 ml of 2% solution of $FeCl_3$ was added in 2 ml of leaf extract was added. The appearance of blue green or black indicated the presence of Phenols and Tannins.

Result and Discussion:-

Qualitative phytochemical analyses of *Lawsoniainermis* L. and *Jatropha gossypifolia* L. have been depicted in Table 1 and Plate 1. Kittiman*et.al*.,2000 reported that 75% of the world's population depends on local health practitioners and traditional medicines for their primary needs. Our phytochemical analysis revealed the presence of the major classes of phytochemicals in ethanolic, methanolic, and aqueous leaf extracts of *Lawsonia* and *Jatrophai.e.* Carbohydrates, Proteins, Phenols, and Tannins, Flavonoids, Saponins, Glycosides, Steroids, Alkaloids and Terpenoids. These phytochemicals are proven to be effective against several diseases, and disorders and conduct certain biological functions that enhance the therapeutic activities i.e., anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha*et.al*.,2020)

These natural products and their derivatives have minimum side effects and improved efficacy than other synthetic counterparts. Alkaloids perform anti-microbial, Cytotoxic, analgesic, and antispasmodic activities (Harborne *et.al*.,1973; Nobori*et.al*.,1994; Antherden*et.al*.,1969; Stray *et.al*.,1998; Okwu*et.al*.,2004). Flavonoids reduce the risk

of Cardiovascular diseases and have tremendous Cholesterol-lowering effects *in vivo*, effective antioxidants, anti-tuberculosis, and anticancer activities, also known as Vit P and Plant Modifier (Ballard and Marostica, 2019; Esmaillzadeh*et.al*.,2006; Veerachari*et.al*.,1998; Askun*et.al*.,2013; Cantrell *et.al*.,2011; Salah *et.al*.,1995; Del-Rio *et.al*., 1997; Okwu, 2004). The Tannins also reduce the risk of heart disease (JanakyRanjithkumar*et.al*.,2010), Anti-oxidant, Anti-microbial, Cytotoxic (Mazni*et.al*.,2016). Raquel (2007) reported that Tannins also bind to proline-rich proteins and interfere with protein synthesis. Similarly, Steroids have analgesic and antibacterial properties and impart in sex- hormones (Okwu,2001).

Saponins are natural glycosides (Irma Podolak*et.al*.,2010) that have an inhibitory effect on inflammation, precipitating and coagulating RBC, haemolytic activity, cholesterol binding properties, and bitterness. (Okwu,2004; Sodipo*et.al*.,2000). The phenolic compounds and phenolic acid are natural antioxidants (Ali *et.al*.,2008) As the use of therapeutic medicine is as old as humans of use of medicinal plants for the prevention of diseases, disorders, and cure of ailments can be traced in Rigveda. Our outcomes of the research will be fruitful for the development of potent herbal drugs against various diseases and disorders in the greater interest of mankind and the environment.

Plate 1

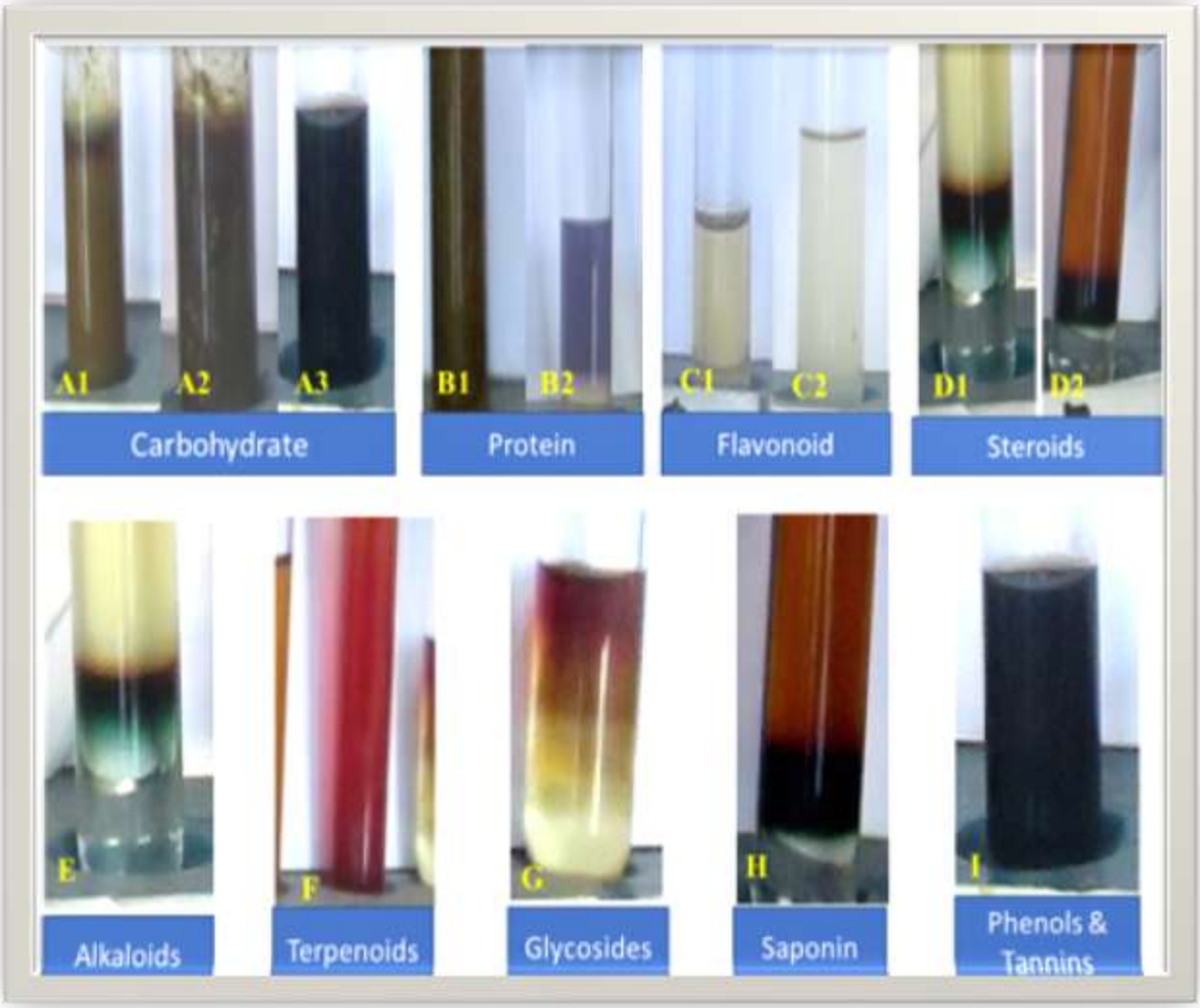

Fig:- Phytochemical analysis of leaf extracts (Aqueous, ethanolic, methanolic) of *L. inermis* L. and *J. gossypifolia* L.

Table 1:- Qualitative Phytochemical analysis of *Lawsoniainermis* L. and *Jatropha gossypifolia* L.

S. No.	Phytochemicals	Test System	L. inermis L.			J. gossypifolia L.		
			A	E	M	A	E	M
1.	Carbohydrate	A1	+	+	+	+	+	+
		A2	+	+	+	-	+	-
		A3	-	-	-	-	-	-
2.	Protein	B1	+	+	+	+	-	+
		B2	-	-	-	+	-	+
3.	Flavonoids	C1	+	-	-	+	-	+
		C2	+	-	+	+	+	+
4.	Steroids	D1	+	+	-	+	-	+
		D2	-	+	+	-	+	+
5.	Alkaloids	E	+	+	+	-	-	+
6.	Terpenoids	F	-	-	-	+	-	-
7.	Glycosides	G	+	-	+	+	+	+
8.	Saponin	H	-	+	+	+	+	+
9.	Phenol & Tannin	I	+	+	+	+	+	+

(+): Present, (-): Absent

A: - Aqueous extracts, E: - Ethanolic Extracts M: - Methanolic Extracts

A1-Fehling's test, A2- Benedict's test, A3- Iodine test, B1-Millon's reagent, B2- Ninhydrin test, C1- Shinoda test, C2- Alkaline reagent test, D1- Chloroform Test, D2- Acetic-acid method, E- Mayer's and Wagner's reagent test, F-Chloroform test, G- Keller-Kilani Test, H- Foam test, I- Ferric chloride test.

References:-

1. Abelson, P. H., (1990). Med. Plants Sci. 247;513.
2. Ali, S. S., Kasoju, N., Lutra, A., Singh, A., Sharanabasava, H., Sahuand, A., Bora, U. (2008). Indian medicinal herbs as source of antioxidants. Food Res. Int., 41: 1-15.
3. Antherden, L. M. (1969). Textbook of Pharmaceutical Chemistry, 8thedn., Oxford University Press, London, pp. 12-16.
4. Askun, T., Tekwu, E.M., Satil, F., Modanlioglu, S., Aydeniz, H., (2013). Preliminary antimycobacterial study on selected Turkish plants (Lamiaceae) against Mycobacterium tuberculosis and search for some phenolic constituent. BMC Complementary and Alternative Medicine, 13(1), 1-11.
5. Ballard, C. R. and Marostica, M. R. (2019). Health benefits of flavonoids.,Bioactive Compounds.
6. Batiha, G.E. and Beshbishy, A. M. (2020). Gas chromatography-mass spectrometry analysis, phytochemical screening and anti-protozoal effects of the methanolic Viola tricolor and acetonic Laurus nobilis extracts, BMC Complementary Medicine and Therapies, 20(87).
7. Bonjean, K., De Pauw-Gillet M C et al., (1998) J. Ethnopharmocol, 69; 241-246.
8. Cantrell, C. L., Franzblau, S. G., Fisher, N. H., (2001). Antimycobacterial plant terpenoids. Planta Medica, 67(08), 685-694.
9. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Micro-Reviews. 12(4):564-582.
10. Del-Rio, A., Obdululio, B. G., Casfillo, J., Main, F. G., Ortuno, A. (1997). Use and propertiesof citrus flavonoids. J. Agric. Food Chem., 45:4505-4515.
11. Dhale, D.A., Birari, A.R., (2010); Preliminary screening of antimicrobial and phytochemical studies of Jatropha gossypifolia Linn. Recent Research in Science and Technology. 2(7):24-28
12. Edeoga, H.O., Okwu, D.E., Mbacbie, B.O. (2005) Phytochemical constituent of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7):685-688.
13. Esmaillzadeh, A., Tahbaz, F., Gaieni, I., Alavi-Majd, H., Azadbakht, L., (2006). Cholesterol lowering effect of concentrated pomegranate juice consumptionin type II diabetic patient with hyperlipidemia. International Journal for Vitamin and Nutrition Research. 76(3), 147-151.
14. Farnsworth, N. R. and Bingel, A. S. (1977). New natural products and plant drugs with pharmacological, biological or therapeutical activity, Springer Berlin, Heidelberg, New York.1-22.
15. Gaikwad, R. S., Kakde, R. B., Kulkarni, A. U., Gaikwad, D. R., Panchal, V. H. (2012) In vitro antimicrobial activity of crude extracts of Jatropha species, Current Botany, 3(3):09-15.

16. Harborne, J. B. (1973). Phytochemicals Methods. Chapman and Hall Ltd., London, pp. 49-188.
17. Nobori, T., Miurak, K., Wu, D. J., Takabayashik, L.A., Carson, D. A. (1994). Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human cancer. Nature, 46:753-756.
18. Irma Podolok, Agnieszka Galanty and Danuta Sobolewska, (2010), Saponins as cytotoxic agents: a review,9(3);425-474.
19. Iwu, M. M., Duncan, A. R., Okunji, C. O. (1999). New Antimicrobials of Plant Origin. In: Perspectives on new crops and new uses. Ed.: Janick J, ASHS Press, Alexandria V A.
20. JanakyRanjithkumar et al., (2010), J. Chem. Pharm Res., 2(4); 371-377.
21. Kirtikar, K.R., Basu, B.D. (1996): Indian Medicinal Plants, International Book Distributors, Allahabad, p. 2247.
22. Kittiman et. al. (2000) Use of plants as medicine under semi and tropical climate of Raichur district of north Karnataka. J. Med. Pl, Science (22-23):406-410.
23. Kosalge, S. B., Fursule, R. A. (2009) Investigation of ethnomedicinal claims of some plants used by tribals of Satpura Hills in India. Journal of Ethnopharmacology.121:456-461.
24. Mazni Abu, Z., ho Yin, W., Azizul, I., and Nurdin, A. (2016). Antioxidant, antimicrobial and cytotoxic potential of condensed tannin from Leucaena leucocephala Hybrid Rending. Food Sci. Hum. Wellness, 5(2); 65-75.
25. Muhammad, H. and Muhammad, S., (2005). The use of L. inermis L. (henna) in the management of burn wound infection. Afr. J. Biotechnology, 4:934-937.
26. Nobori, T., Miurak, K., Wu, D. J., Takabayashik, L. A., Carson, D. A. (1994). Deletion of cyclin-dependent kinase-4 inhibitor gene in multiple human canaers. Nature, 46; 753-756.
27. Okwu, D. E. (2004). Phytochemicals and vitamins content of indigenous species of southeast Nigeria. J. Sustain. Agric. Environ., 6(1): 30-37.
28. Okwu, D. E., Okwu, M. E. (2004). Chemical composition of Spondiasmombinlinn. Plant parts. J. Sustain. Agric. Environ., 6(2): 140-147.
29. Okwu, D. E. (2001). Evaluation of chemical composition of medicinal plants belonging to Euphorbiaceae. Pak Vet. J., 14:160-162.
30. Raja Sekaran, P. E., (2001). Medicinal Plant Sector in India. 23-31.
31. Ram, S. and Sinha, V. S., (2017), "Comparative Qualitative Phytochemical Analysis of seven plants belonging to family Caesalpiniaceae with special emphasis on Quinine", International Journal of Current Advanced Research, 6(5); 3652-3655.
32. Raquel, F. E. (2007). Bacterial lipid composition and antimicrobial efficacy of cationic steroid compound. Biochemica et Biophysica Acta. 2500-2509.
33. Saini, V., Mishra, R., Mandloi, S., Yadav, N. (2015), Analysis of the phytochemical content of J. gossypifolia L. Chemical and Process Engineering Research Vol 3 (5)99-105.
34. Salah, N., Miller, N.J., Pagange, G., Tijburg, L., Bolwell, G. P., Rice, E., Evans, C. (1995). Polyphenolic fflavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. Are. Biochem. Broph.,2:339-346.
35. Sodipo, O. A., Akiniyi, J.A., Ogunbamosu, J. U. (2000) Studies on certain characteristics of extracts of bark of Pansinystaliamacruceras (K schemp) picrreExbeille. Global J. Pure Appl. Sci., 6:83-87.
36. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp.191-289.
37. Stray, F. (1998). The Natural Guide to Medicinal herbs and Plants. Tiger Books International, London, pp.12-16.
38. Tanaka, H., Sato, M., Fujiwara, S., Hitara, M., Etoh, H., Takeuchi, H., (2002), Letters in Applied Microbiology, 35(6): 494-498.
39. Tudu, D. and Sinha, V. S. (2015). Medicinal Plants of West Singhbhum commonly used by Tribes to cure Anemia. International journal for Exchange of Knowledge; 2(1): 74-80.
40. Tudu D and Sinha V S. (2017). An Ethnobotanical survey on medicinal plants used to mitigate anemia by tribes of east and west Singhbhum districts of Jharkhand, India. Journal of Pharmacognosy and Phytochemistry, 6(6), 2592-2595.
41. Veerachari, U. and Bopaiah, A. K., (2011), Preliminary Phytochemical evolution of leaf extracts of five Cassis Species, J. Chem Pharm. Res.,3(5);574-583.