International Journal of Applied and Universal Research **E-ISSN No: 2395-0269**
Volume X, Issue III, May – June 2023 **Available online at: www.ijaur.com**
Impact Factor- 5.937

PHYTOCHEMICAL ANALYSIS AND SCREENING OF TOTAL FLAVONOID, TANNIN AND PHENOLIC CONTENTS IN CROTON SPARSIFLORUS MORONG

Sushma verma[1], Dr. Pramod Kureel[2] and Dr. (Smt.) Sonu Rahi[3]
1. Research Scholar Botany Govt. Girls P.G. College, Rewa (M.P.)
2. Ortho & Sports Physiotherapist, Govt. Ayurved College, Rewa (M.P.)
3. Asstt. Prof. of Botany, Govt. Girls P.G. College, Rewa (M.P)

ABSTRACT :- The aim is to study the phytochemical constituents present in Croton sparsiflorus leaf and stem. Preliminary phytochemical analysis revealed the presences of phytochemicals such as carbohydrates, quinines and glycosides in all the tested extracts in both the leaf and stem of Croton sparsiflorus g. Coumarins, proteins, steroids, phytosteroids, phlobatannins and anthraquinones were absent in all the tested extracts. The amount of total phenolics, total flavonoids and tannin content in hexane, ethyl acetate and methanol extracts of leaf and stem were determined spectrometrically. The hexane, ethyl acetate and methanol extract of stem showed higher total phenolic, flavonoid and tannin content then leaf. The present study suggested that, detailed studies on the isolation and characterization of the leaf and stem extracts as well as investigations on other biological studies and in vivo assays will be interesting in discovering new drugs.

KEYWORDS:- Croton sparsiflorus Morong, phytochemical screening, medicinal uses.

INTRODUCTION :-

Traditional folk medicine involves use of herbal and natural products to treat various diseases. Reports from WHO states that 70- 80% of the total population in the world use herbs as alternative medicine (Divya *et al*., 2011). In India, over 7500 plant species are being used in traditional medicines (Singh *et al*., 2011). The plant constituents are classified as primary and secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds were often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites. Secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases.

Euphorbiaceae family in the plant kingdom is a complex hetero-geneous family consisting of about 322 genera and 8900 species in the world. *Croton sparsiflorus Morong* belonging to Euphorbiaceae family is a native of southern Bolivia, Paraguay, Southwestern Brazil, and Northern Argentina (Chakrabarty and Balakrishnan, 1992). In India, *Croton sparsiflorus Morong*, commonly called as "Railpachilai" in South and "Ban Tulsi" in North are found abundant in Malda, West Bengal and Assam (Mahadeswara Swamy, 2006). Its stem and bark paste is used for treating skin diseases, treat headaches and to arrest bleeding wounds (Balasubramanian *et al*., 1997, Ajit *et al*., 2007, J. Lenin and Venkat., 2009). Stem and branches are used as fuel and its ashes are used as detergents in interior eastern India (Mahadeswara Swamy, 2006). On the other hand, seeds are used for the treatment of jaundice, acute constipation abdominal dropsy and internal abscesses (Divya *et al*., 2011).

Croton sparsiflorus Morong (Family- Euphorbiaceae) is a small annual herb, growing mainly road side up to 1-2 ft tall. Alternately arranged leaves, 3-5 cm long, are lance-shaped, with toothed margin. Small white flowers are borne in 3-8 cm long racemes at the end of branches. Flowers have 5 sepal and 5 petals and numerous long stamens producing out. Fruit is 5mm oblong capsule with warty surface. The plant is well known under vernacular as "Ban Tulasi" The powdered leaves are

useful in controlling high blood pressure and used for treatment of skin disease, cuts & wounds as well as antiseptic and antidote (Nishanta et.al. 2002 ; Chaudhari AB. 2007 & Bhakat RK. 2008). It contains broad spectrum antibiotic compounds in leaves of this species (Srinivasan et. al. 2001). This plant main chemical constituents i.e. glycoside, saponins tannins, flavonides, terpenoids and alkaloids (Okeke et. al. 2001 & Shamala et.al; 2009).

Fig. 1: The plant of *Croton sparsiflorus.*

The most of phytoconstituents were extracted from leaves of C. sparsiflorus Morong, hence the leaves of this plant have been used for all pharmacological activities.

AIMS AND OBJECTIVE:-

The aims and objective of the study phytochemical constituents present in Croton sparsiflorus leaf and stem. Preliminary phytochemical analysis revealed the presences of phytochemicals such as carbohydrates, quinines and glycosides in all the tested extracts in both the leaf and stem of Croton sparsiflorus g. Coumarins, proteins, steroids, phytosteroids, phlobatannins and anthraquinones were absent in all the tested extracts.

REVIEW OF LITERATURE:-

In India some ecological studies have been made for few medicinal plants including morphology, ecology study, reproductive biology etc. Udayaprakash, et al. (2011), Saranya, et al. (2012), Shende, et al. (2015), Duraipandyan, et al. (2011), Ramakrishnan, et al. (2012), Mitra and Nayar (2017), Khanna. (2017), Reddy, et al. (2017), Reddy, Saini and Sihag (2020), Akkulanna and Kailas (2020), and Renu and Nahid (2020).

MATERIALS AND METHODS:-

Collection and Identification of Plant Material:

The entire plant of Croton sparsiflorus Morong were collected from Rewa Madhya Pradesh India in May, 2022. The botanical identity of the plant was JNKVV Agriculture College Rewa (M.P).

Extraction and Preparation of the Extract:

After collection, the plant materials were air dried for one week. This was further subjected to another one week of drying in an oven maintained at 400C. The leaves were pulverized into a smooth powder. The pulverized plant material (150g) was mixed with distilled water (3.0 liters) and left for 72 hours. The mixture was stirred at 6 hours intervals using a sterile glass rod. At the end, the extract was passed through filter paper. The filtrates were concentrated with the aid of a vacuum pump and rotavapour at 400 C. The concentrated extract was stored in cool places prior to use.

Phytochemical Screening:

The aqueous extract was subjected to phytochemical screening testing for the presence of alkaloids, Tannins, saponions, reducing sugars and carbohydrate using the method of Trease and Evans. The aqueous extract (4 g) was warmed with water on a steam bath for 30 min then filter. After filtration, the filtrate obtained was tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent (Trease, G.E et. al. 1989 & Harborne J B, 1984).

RESULTS AND DISCUSSION:-

Results of different chemical tests on the ethanolic extract of *C. sparsiflorus* stem & leaf showed the presence of alkaloid, tannin, steroid etc. (**Table 1**).

Table 1: Results of different group tests of aqueous extract of *C. sparsiflorus* Morong

Phytoconstituents	Results
Alkaloids	Present
Reducing sugars	Present
Flavonoids	Present
Saponins	Absent
Steroids	Present
Gums	Absent
Tannins	Present
Carbohydrates	Present

The preliminary phytochemical analysis of Hexane, Ethyl acetate and Methanol extracts of *Croton sparsiflorus Morong* leaf samples shows the strong presence of Carbohydrates, Tannins, Flavonoids and Phenols in all the three extracts, except Diethyl ether showed Carbohydrates and Phenols. There is a sparse presence of alkaloids and glycosides in the ethyl acetate and methanol extracts. Saponin, coumarins and steroids are present in the methanol extract. The phytocompounds quinones, glycosides, terpenoids, triterpenopids, phlobatannins and anthraquinones are absent in all the four extracts. Similarly the preliminary phytochemical analysis of the stem samples shows the strong presence of carbohydrates, tannins, flavonoids and phenols. Alkoloids and steroids are present in the hexane and ethyl acetate samples. Saponins are present in the hexane and methanol samples. The other phytochemicals namely quinones, glycosides, terpenoids, triterpenopids, phlobatannins and anthraquinones are absent in stem and leaf. Based on the presence of phytocompounds the further estimation will carried out with three solvent Hexane, Ethyl acetate, and methanol.

As phytochemicals often play an important role in plant defense against prey, microorganism, stress as well as interspecies protections, these plant components have been used as drugs for millennia and hence, screening of phytochemicals serves as the initial step in predicting the types of potential active compounds from plants (Chew *et al*, 2011).

Flavonoid compounds especially quercetin and genistein have antitumor activity and these compounds are cytotoxic to cancer cells but have no or insignificant activity in normal cells (Pouget *et al*, 2001). It has been reported that flavonoid, apigenin holds great promise as a chemopreventive agent for a variety of cancers and exhibits significant activity against UV induced DNA damage and thus protect against skin cancer (Baliga and Katiyar, 2006). Plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (Polterait, 1997).

These compounds present in a variety of medicinal plants have significant application against human. Pathogens, including those that cause enteric infections and are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan *et al*., 2004). Alkaloids are formed as metabolic byproducts and have been reported to be responsible for the antibacterial activity in most of the medicinal plants (Mantle *et al*, 2000). Glycosides serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Dhar *et al*, 1979).

Estimation of Total phenolic content (TPC)

The concentrations of total phenolic content in the extracts were expressed as mg of Gallic acid equivalent per g of dry weight (mg GAE/g) of extract the total phenolic content of the leaf and stem samples of *Croton sparsiflorus Morong* were determined using the Folin-Ciocalteu reagent method. The reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm was measured spectrophotometrically. The results revealed the presence of highest total phenol content in the hexane extract of the leaf in *Croton sparsiflorus Morong* is 14µg/g whereas the total phenol content in the methanol extract of the stem is 28µg/g. The methanol

and ethyl acetate leaf extracts contain the total phenol of 13µg/g and 12µg/g respectively. The hexane and ethyl acetate stem extracts contain 10µg/g and 20µg/g of total phenol content. Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenols are able to scavenge reactive oxygen species due to their electron donating properties (Re *et al*., 1999 and Velioglu *et al*., 1998).

Estimation of Total flavonoid content

Total flavonoid content of samples was obtained in comparison with the Quercitin standard. The total flavonoid content of the leaf and stem samples of *Croton sparsiflorus Morong* were determined by aluminum chloride method. The results made known the presence of highest flavonoid content in the ethyl acetate and methanol extracts of the leaf to be 14µg/g, whereas the ethyl acetate extract of the stem contains the highest flavonoid content of 65µg/g. The hexane extract of the leaf contains 12µg/g, the hexane and methanol extract of the stem contains 16µg/g and 56µg/g of total flavonoid. The results indicate that the stem of *Croton sparsiflorus Morong* contains more flavonoids than the leaf.

Estimation of total Tannin

The total tannin content was expressed as mg of Tannic acid equivalent/g of dry weight (mg E/g) of extracts. The total Tannin content of the leaf and stem samples of *Croton bonplandianum* were determined using leaf extracts measured by Folin–Denis method. The results specify that the total tannin content in the methanol extract of the leaf is 68µg/g, whereas the ethyl acetate and the methanol extracts contain 60µg/g and 48µg/g of tannin content. Comparatively the methanol extract of the stem contains 249µg/g, the hexane and methanol extracts contain 91µg/g and 231µg/g of tannin respectively. The results designate that the stem of *Croton sparsiflorus Morong* contain more tannin when compared to the leaf.

However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principle(s) and exact mechanism(s) of action.

FINDING: -

Medicinal plants are used since ages by the primitive society and many of them have figured in Ayurvedic literature also. The present study will provide mankind a medicine which is safe and free from side effects. The results indicate that the plant contains numbers of secondary metabolites and significant against traditional uses. The present study of the phytochemical screening, total phenolics, total flavonoids and total tannins of different extracts in *Croton sparsiflorus Morong* leaf and stem showed that, these plants could be a potential source for natural antioxidants. It has been reported that most active principles in *Croton sparsiflorus Morong* are frequently alkaloids, flavonoids and phenols and these may be responsible for many of the pharmacological actions of the particular plant.

CONCLUSION:-

The results revealed the presence of medicinally important constituents in the *Croton sparsiflorus*. Biological studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the *Croton sparsiflorus*. Therefore, extracts and isolated compounds from *Croton sparsiflorus* could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for *Croton sparsiflorus*. It is hoped that the strong knowledge of natural products coupled with combinatorial sciences and high-throughput screening techniques will improve the ease with which natural products and formulations can be used in drug discovery campaigns and development process, thereby providing new functional leads for various diseases. If these plants are examined for further biological studies, it could be a promising agent in scavenging free radicals and treating diseases related to free radical reactions. Furthermore, detailed studies on the isolation and characterization of the plant extract as well as studies with other models such as lipid peroxidation and *in vivo* assays will be interesting in discovering new drugs.

REFERENCES:-

1. Ajit K.D., Dutta. B. K and Sharma. G. D (2007) "Medicinal plants used by different tribes of Cachar

district, Assam". *Indian Journal Of traditional Knowledge* , Vol7(3), pp 446-454.

2. Akkulanna, S., Kailas, Jangam Ganga, Kullayiswamy, k. Raja, Raju, R.R. Venkata and Ramakrishna, Hari (2020). Pollen diversity in Phyllanthus species (phyallanthaceae) Andhra Pradesh, India. Agricultural Science Digest,4(4): 350-356.
3. Balasubramanian.P, Rajasekaran.A and Prasad.S.N (1997) "Folk Medicine Of The Irulas of Coimbatore Forests". Ancient Science of Life Vol XVI 3, pages 222 – 226.
4. Baliga, MS & Katiyar, SK 2006, Chemoprevention of photocarcinogenesis by selected dietary botanicals, Photochemical and Photobiological Sciences, 5: 243–253.
5. Bhakat RK, Sen UK. (2008) Ethnomedicinal plant conservation through sacred groves. Tribes and Tribals 2008; 2: 55-58
6. Chakrabarty, T. and Balakrishnan, N.P. (1992). A revision of *Croton* L. (Euphorbiaceae) for Indian subcontinent. Bulletin of the Botanical Survey of India 34(1-4): 1- 88.
7. Chaudhari AB. (2007) Special habitats and threatened plants of India, Endangered medicinal plants. 1st ed. Daya publishing house New Delhi, 2007, pp. 312.
8. Chew, YL, Chan, EWL, Tan, PL, Lim, YY, Stanslas, J & Goh, JK 2011, Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia, BMC Complementary and Alternative Medicine, 11: 12.
9. Dhar, ML, Dhar, MM, Dhawan, BN & Ray C 1979, Screening of Indian plants for biological activity. Part-I, Indian Journal of Biology, 6: 232-234.
10. Divya. S., Naveen. K.K., Ramachandran. S and Dhanaraju. M. D. (2011) "Wound Healing and In Vitro Antioxidant Activities of Croton bonplandianum Leaf Extract in Rats". Global Journal of Pharmacology 5(3): 159-163.
11. Duraipandyan, and Ignacimuthu, S. (2011). Antifungal activity of traditional medicinal plant from Tamil Nadu, India, Asian pacific Journal of Tropical Biomedicine S204-S215.
12. Harborne J B, (1984) Phytochemical Methods (A Guide to Modern technique to plant Analysis) 3rd Edn. Chapman and Hall, London, pp. 132-324.
13. Hassan, MM, Oyenwale, AO, Abduallahi, MS & Okonkwo, EM 2004, Preliminary Phytochemical and Antibacterial investigation of crude extract of the root bark of *Datarium microcarpum*, Journal of Chemical Society Nigeria, 29: 26-29.
14. Khanna, K.K. (2017). Angiospermic plants of Uttar Pradesh - A Check – list. Geophytology. 47(1): 69-110.
15. Lenin B.J and Venkat R.S. (2009). "Traditional Uses of Some Medicinal Plants by tribals of Gangaraju Madugula Mandal of Visakhapatnam District, Andhra Pradesh". *Ethnobotanical Leaflets* 13: 388-98.
16. Mahadeswara Swamy (2006) Ban Tulsi (Online), Available: http://www.flowersofindia.net/catalog/slides/Ban%20Tulsi.html (25 Jul, 2006).
17. Mantle, D, Eddeb, F & Pickering, AT 2000, Comparison of relative antioxidant activities of British medicinal plant species in vitro, Journal of Ethnopharmacology, 72.
18. Mitra, R.L. and Nayar, M.P. (2017). Phyllanthus airy-shawii Replace Phyllanthus debilis (Euphorbiaceace) of the flora of British India. International association for Plant Taxonomy. 37 (2): 469-470.
19. Nishanta R, Harris CS, Towers GHN (2002), Antimicrobial activity of plants collected from Serpentine outcrops in Sri Lanka. Pharmaceutical Biology; 40(3): 235-244 http://dx.doi.org/10.1076/phbi.40.3.235.5825
20. Okeke MI, Iroegbu EN, Eze EN, Okoli AS, Esimone CO. (2001)- Evaluation of extracts the roots of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol 78: 119-127. http://dx.doi.org/10.1016/S0378-8741(01)00307-5
21. Polterait, O 1997, Antioxidants and free-radical scavengers of natural origin, Current Organic Chemistry, 1: 415-440. .

22. Ramakrishnan, S., Dhanavathy, R., M., Lakshmi, Thilagam M., Sivagamy, D. and Prema, R. (2012). Ethanolic extract form fresh leaf of Croton sparsiflorus Morang exhibited antibacterial activity against four gram-negative bacteria and histochemical studies. Journal of pharmacy Research, 5: 2201-2203.

23. Re. R, Pellegrini. N, Proteggente. A, Pannala. A, Yang. M and Rice-Evans. C. (1999). Antioxidant activity applying animproved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*; 26: 1231–1237.

24. Reddy, B.S., Rao, N.R., Vijeepallam, K., and Panday, V. (2017). Phytochemical, pharmacological and biological profiles of Tragia species (family: Euphorbeace). Afr. J. Tradit, Complement. Alt. Med., 14 (3): 105-112.

25. Renu, Satya Narain and Nahid, F animals 2020. Tragic plukenetii radcl.-Sm.(Euphorbiaceae): An Addition to Flora of Allahabad, Uttar Pradesh, Current Botany,11: 36-37.

26. Saini, Reena and sihag, Ram Chander (2020). Flowering Biology and Phenology of Aonla (Emblica officinalis Gaertn.) in the Semi-arid Environment of North-West India. International Journal of Agricultural Research, 15: 1-8.

27. Sakanaka, S, Tachibana, Y & Okada, Y 2005, Preparation and antioxidant properties of extract of Japanese persimmon leaf (kakinoha-cha), Food Chemistry, 569-575.

28. Saranya, S., Anita, D., Vennila, D., Hematite, A., Kuppamma, K. and Ramakrishnan, S. (2012). N-Butanol and Iso- Propanol Extract from Croton sparsiflorus Exhibited Antibacterial Property and Histochemical Studies in Stem and Root. Journal of Pharmacy Research, 5: 2212-2214.

29. Shamala GowriS, Vasantha K.(2009) Phytochemical and comparative antibacterial studies on the crude hexane and aqueous extracts of the seed of *Harpullia arborea* Radlk. Plant Archives; 2: 903-904.

30. Shende, M.B., Deshmukh, U.B. and Rathor, O.S.(2015). A Preliminary Survey of Cyanogenic Plant of Family Euphorbiaceae from Chandrapur District of Maharashtra (India), BJR 2(6): 173-179.

31. Singh, B., Dutt, N., Kumar, D., Singh, and Mahajan, R. (2011). Taxonomy, Ethnobotany and Antibacterial Activity of Croton bonplandianum, Euphorbia hirta and phyllabthus fraternus. Journal of Advanced Developmental Research 2: 21-29.

32. Singh.B.,Dutt.N., Kumar.D., Singh.S. and Mahajan R. (2011) "Taxonomy, Ethnobotany and Antimicrobial Activity of *Croton bonplandianum*, Euphorbia hirta and Phyllanthusfraternus". Journal of Advances in Developmental Research 2(1): 21-29.

33. Srinivasan D, Perumalsamy LP, Natahn S, Sures T. (2001) Antimicrobial activities of certain Indian medicinal plants used in folkloric medicine. J Ethnopharm 2001; 94: 217-222. http://dx.doi.org/10.1016/S0378-8741 (00)00345-7

34. Trease, G.E and W.C. Evans, (1989). Pharmacognosy. 13 Edn. English Language Book Society, Bailliere Tindall, Britian, pp. 386-480.

35. Udayaprakash, N.K., Bhuvaneswari , S., Aravind, R., Kaviyarasan, v., Kalaivanan, K., Hariram, S.B. (2011). A comparative study on Antibacterial activity of common weeds, Internation Journal of Pharma and Bio Sciences, 2: 677-683.

36. Velioglu. Y. S, Mazza. G, Gao. L and Oomah. B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grainproducts. *Journal of Agricultural Food & Chemistry*; 46: 4113–4117.