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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY STUDIES OF PLANT *Anisomeles Indica* FOR DETECTION OF VARIOUS NATURAL BIOACTIVE COMPOUNDS

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ABSTRACT

HPLC chromatogram gave different peaks of several compounds present in stem of plant extract. Thus, based on these analytical results, it was concluded that the ethanolic extractof Anisomeles indica stem have rich amount of phytochemical compounds that are significant source for the production of latest drugs for inhibition of various ailments. Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities. The pharmacological activity of a plant can be predicted by the identification of the phytochemicals. Currently, phytochemical screening of ethanolic extract of Anisomeles indica stem was determined by using standard procedures. HPLC analytical technique is also used for the detection of various natural bioactive compounds.

Keywords: Acalypha indica, HPLC Medicinal plants, Phytochemical screening,

INTRODUCTION

The medicinal properties of the plants are determined by the phytochemical constituents (Ezeonu and Ejikeme, 2016). Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed in various parts of the plants (Sheelet al., 2014). Phytochemicals are chemical compounds naturally present in the plants, which have either defensive or disease protective propertsies (Silvaet al., 2017). The standardized extracts of medicinal plants, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Coset al., 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Boligonand Athayde, 2014). Nature is a unique source

of structures of high phytochemical diversity representing phenolics (45%), terpenoids and steroids (27%)and alkaloids (18%) as major groups of phytochemicals (Saxena et al., 2013). Although, these compounds seem to be non-essential to the plant producing them, they play a vital role in survival by mediation of ecological interactions with competitors, protect them fromdiseases, pollution, stress, UV rays and also contribute for color, aroma and flavor with respect to the plant (Shaikh and Patil, 2020). The metabolites produced by the plants to protect themselves against biotic and abiotic stresses have turned into medicines thatpeople can use to treat various diseases (Kocabas, 2017).

The extract of dried leaves helps in dermal wound healing as itelevates the synthesis of collagen due to its antioxidant activity (Ganesh kumar et al., 2012). Anisomeles indica belongs to the family is one of the medicinal herbs widely distributed in Malaysia. All parts of the plants have medicinal values such as the flower thatcontain flavonoids (Nahrstedtet al., 2006) as well as the leaves and twigs that contain acalyphamide (Deep, 2001). Other than that, the root of the plant has antihelminthic properties (Chengaiahet al., 2009) while the whole plant extract possesses antiarthritic activity (Krishnaet al., 2011). Badami andChannabasavaraj, 2007 reported antioxidant properties of methanolic extract of stem and Selvamani, 2015 revealed with acetone extracts. A.indica leaf, root and stemextracts/fractions scavenge the hydrogen peroxide, superoxide radicals, nitric oxide,and metal ions (iron and molybdenum); protect the hydroxyl radicals induced sugar damage and lipid peroxidation (Badami and Channa basava raj, 2007; Balakrishnanet al., 2009; Sanseeraet al., 2012; Rani, 2014; Selvamani, 2015; Raviet al., 2015; Raviet al., 2017). The methanolic extract of A. indica leaves, stem and roots showed maximum activity against Candida albicans (Raviet al., 2021).Even though it has been used traditionally in treating ailments but to date, limited scientific data has been revealed about stem part of this medicinal herb. Therefore, this present study was carried out to analyzing the phytochemicals present in stem extract of A. indicathrough the chromatographic technique.Because plants are always used for curing the disease from the ancient time hence it is important to analysis of chemical present in plant parts (Tripathiet al., 2012).

MATERIAL AND METHOD

1) COLLECTION OF PLANT MATERIALS

Fresh stem of Anisomeles indica was collected from Botanical garden of the Glocal University, Uttar Pradesh, India. The stem was thoroughly washed under running tap water to remove debris and the leaveswere shade dried at room temperature for 14 days. The dried samples were pulverized to powder using mortar-pestle and stored in polythene bag. Approximately 8kg of the powdered plant material was extracted by cold maceration method with ethanol left for 72 hours with intermittent shaking. The plant extracts was filtered and then concentrated using rotary evaporator at 40^{o}C, and each extract was transferred into well labelled sterile glass vials and stored at 4^{o}C before use.

2) PHYTOCHEMICAL SCREENING

Phytochemical Screening was carried out on the ethanolic extracts of A. indica stem using standard protocols (Trease and Evans, 1989; Sofowora, 1993; Ejikemeet al., 2014; Kokateet al., 2004) for the identification of phytochemicals compounds such as Tannins, Saponins, Flavonoids, Terpenoids, Steroids, Alkaloids, Cardiac Glycoside, Phenols, Fatty Acids, Resins and Triterpenoids.

3)HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High pressure liquid chromatography is a technique performs in order to confirm theactive phytochemical compounds in the ethanolic extracts of A. indica stem. An isocratic HPLC (Shimadzu HPLC class VP series) with two LC-10 AT VP pumps (Shimadzu), variable wavelength programmable photodiode array detector SPD MIOA VP (Shimadzu), CTO-IOASVP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and reverse phase Luna 5 mC18 (2) Phenomenex column (250 mm x 4.6 mm) was used. The HPLC system was equipped with software class VP series version 6.1 (Shimadzu). Chemical separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. The mobile phase components (70% Methanol: phosphate buffer (pH 7.2): ultrapure water in the ratio of 70:4:26)were filtered through a 0.2 µ membrane filter before use and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min which yielded column backpressure of 16-165 Kgf cm^{-2}. The column temperature was maintained at 27ºC. 20 µl ofsample was injected using a Rheodyne syringe (Model 7202, Hamilton) (López-Santigoetal., 2014).

RESULTS AND DISCUSSION

1) Phytochemical Screening

In this study the ethanolic stem extract of A. indica showed the presence of low (+)phlobatannins, flavonoids, terpenoids, steroids, resins, and triterpenoids and absent (-) of alkaloids, phenols, and fatty acids.The saponin was highly (+++) present and tannin was moderately (++).The preliminary phytochemicals screening of ethanolic extracts of A. indicastem were carried out by observing different color reaction that reflects the presence of compounds (Hardainiyanet al., 2015) and these results have been summarized in the table-1. Bioactive natural products have enormous economic importance as specialty chemicals as they can be used as drugs, lead compounds, biological or pharmaceutical tools, feedstock products excipients and nutraceuticals (Pieters andVlietinck, 2005).Nazriet al., 2016 reported moderately presence of triterpenes and steroids and slightly presence of flavonoid in A. indica dried stem.Thequalitative phytochemical study ofTasmimet al., 2021 on ethanolic extract of A. indica stem recognized saponinand flavonoid in moderate amount and alkaloid, phenol, tannin and terpenoid in trace amount.

Table 1: Phytochemical screening of ethanolic extract of Acalyphaindicastem

Phytochemical constituents	A. indica stem
Tannin	++
Saponin	+++
Phlobatannins	+
Flavonoids	+
Terpenoids	+
Steroids	+
Alkaloids	-
Cardiac Glycoside	+
Phenols	-
Fatty acids	-

Resins	+
Triterpenoids	+

2) HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

For standardization of ethanolic extract of plant stem, HPLC is a sensitive and accurate tool that widely used for the quality assessment of plant extract and its derived product/formulation (Jain et al., 2011). HPLC chromatogram of Acalyphaindicastem extract was used for the identification and quantitative analysis of compounds given in Figure 1. 13compounds have been identified in the ethanolicextract of A. indicastem by HPLC analysis. Compositional analysis of stem ethanol extract by HPLC revealed the presence of various peaks at different retention times 0.072, 0.598, 0.682, 3.453, 3.787, 3.955, 10.218, 15.863, 18.997, 22.653, 23.275, 33.885 and 41.257. In which the compound having retention time (RT)3.453 was the main constituents in ethanolic stem extract.The maximum area percent (73.379) was recognized with a compound that showed 3.453 retention time, followed by 3.955 RT exhibited 10.372 area%. Similarly in the methanolic extract of Eucalyptus leaves showed the various constituents with different retention times (2.5500, 3.1393, 3.5000, 4.9000, 5.6333, and 7.0000). The compound that having 2.5500 RT,was the main constituents in methanolic leaf extract (Tripathiet al., 2012).Thus, HPLC is an effective technique to characterization of numerous reported and novel compounds present in ethanolic extract of A. indica.

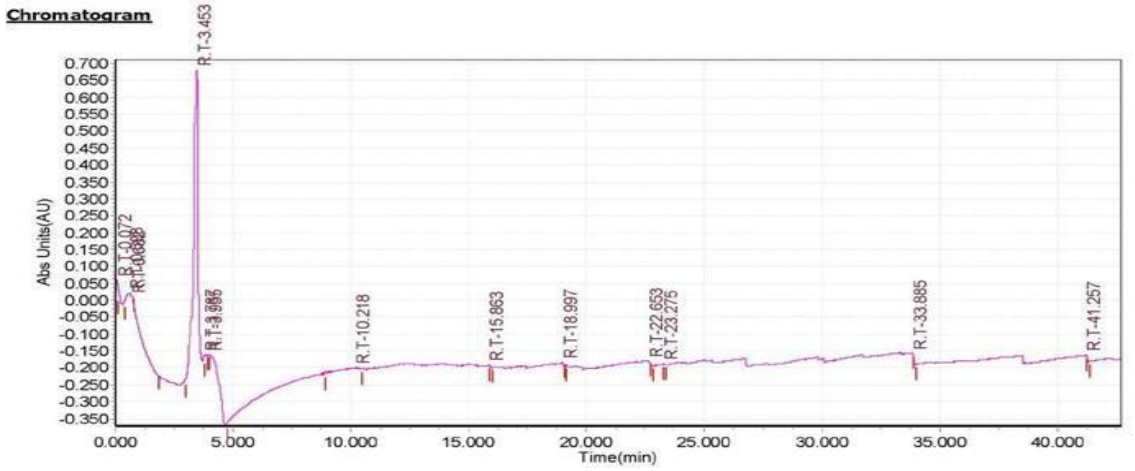

Figure 1: HPLC Spectra of ethanolic extract of A. indica stem

CONCLUSION

Phytochemical screening of the stem part of Acalyphaindica revealed the presence of saponnins, tannin, phlobatannins, flavonoids, terpenoids, steroids and resins which are important secondary metabolites. Phytochemical analysis is very important laboratory process or scientific process. This process is identified

essential components of any plant part such as bark, leaves, stem, and root. Hence it is essential to analyze the phytochemicals present in the plant through a potential technique along with screening method. Based upon the HPLC technique, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in the ethanol extract of plant stem. In conclusion, this study indicates that the phytochemicals in Acalyphaindica provides supporting evidence regarding medicinal values of this herb thus can be an alternative treatment for certain diseases.

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