Comparative Study of Secondary Metabolites from the Leaves of *Euphorbia Hirta* L. and *Acalypha Indica* L. For Their Anticancer Properties

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Abstract

Plants are a great source of biologically active substances such as secondary metabolites can be used for therapeutic, commercial and pharmaceutical products. This study was conducted to evaluate the leaves of *Euphorbia hirta* L. and Acalypha *indica* L. for their anticancer properties on the basis of secondary metabolites as an antioxidant. Estimation of secondary metabolites including parameters such as estimation of Total phenol, Ortho-dihydric phenols, Bound Phenols, Tannins, Quinones and Total Flavonoid was carried out by using spectrophotometer. For this study leaves of *Euphorbia hirta* L. and Acalypha *indica* L. were selected. The amount of total phenol, ortho-dihydric phenols, Bound phenols, Tannins, Quinones and Total Flavonoid of the leaves of Euphorbia *hirta* L. were 20µg/g; 15µg/g; 140µg/g, 45µg/g, 2,780µg/g, and 580µg/g. The amount of total phenol, ortho-dihydric phenols, Bound phenols, Tannins, Quinones and Total Flavonoid of the leaves of Acalypha *indica L.* were 140µg/g; 15µg/g; 300µg/g; 50µg/g; 14,400µg/g; 740µg/g. The presence of phenols, flavonoids, tannins and quinone in both plants exhibit the antioxidant, anticancer, anti-viral activity, anti-tumor, anti-inflammatory, anti-diabetic properties.

Key words: Secondary metabolites, antioxidant, *Euphorbia hirta, Acalypha indica*

I. INTRODUCTION

India is known as the World's "Botanical Garden" and it is the world's second largest producer of medicinal plants. Plants are considered to be precious and natural treasures to providing most dominant natural source of nutrients and therapeutic agents. Throughout human history plants have been found major source of medicines and have great importance by reason of their nutritive value in [1]. Plants are thought to be primary sources of antioxidant compounds that play a crucial role for good health maintenance [2].

In biological system cells can damage the DNA and lead to the oxidation of lipid and proteins by the reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species (ROS) include a variety of free radicals like superoxide anion ($O2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), and peroxyl radical ($RO2^{\bullet-}$), and non-free radical species such as hydrogen peroxide (H2O2) [3].

Nonetheless excessive production of ROS and RNS induced by the exposure of cigarette, radiation, smoking, alcohol and environmental toxins, It like hydroxyl, superoxide and nitric oxide radicals. Naturally, human body can scavenge these radicals by antioxidant system occurring in human body, which helps to keep the balance between oxidation and anti- oxidation. Intake of exogenous antioxidants would improve

the damage generated by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction. Acting as free radical scavengers as well as quenchers of singlet oxygen and reducing agents [4].

Acalypha indica L. and *Euphorbia hirta* L. both are the genera of family Euphorbiaceae. *Acalypha indica* L. commonly known as Indian copper leaf, Kuppi, Kupi, Khokali. It is 30-60 cm tall, annual erect herb. Fresh juice of *Acalypha indica* plant used to treat bronchitis [5]. treating ear aches and oral diseases in Nepal [6]., root decoction used to cure diarrhea [7]., fever [8]., for low blood sugar [9] and as a laxative [10;11;12]. inflammatory, regulating urine (diuretics), laxatives, and stopping bleeding (hemostasis) [13]. *Euphorbia hirta* L. commonly known as dudhi, dudhani, Asthma weed. Among the practitioner of herbal medicine it is very popular shows significant inhibition of the survival of breast cancer cell [14]. *E. hirta* has free radical scavenging activity, and display protection against oxidative damage to protein. [15].

Generally, Traditional medicine practitioners use the aqueous extracts of various *E. hirta* parts and these extracts was used to cure verious diseases, including bowel disease, cough, kidney stones, hay asthma, worm infestation, and bronchial disease, decrease lactation. they have also been utilized for their analgesic, antipyretic, sedative, anxiolytic, and anti-inflammatory properties [16;17]. The whole plant of *E. hirta* has been investigated to exhibit 45% immunomodulation activity by inhibiting NO production [18]. Previous verious research have performed to characterize the chemical compounds present in *E. hirta* [19;20]. But there is a lack of information relating to the amount of phenolic compounds such as bound phenol, ortho-dihydric phenol, flavonoids, tannin and qinone in *E. hirta*. The aim of the present study was to estimate the secoundary metabolites in the leaves *E. hirta* and to evaluate its potential for clinical use as a natural antioxidant and anticancer agent.

II. MATERIALS AND METHODS

Plant material

Euphorbia hirta L. and *Acalypha indica* L. plants were collected from govt. Vidarbha institute of science and humanities, Amravati, Maharashtra, India. The identification of the plants were done on the basis of morphological characters by well-known taxonomist and also cross checked by using standard floras [21;22;23;24]. at Department of Botany, GVISH College, Amravati, Maharashtra, India.

Preparation of plant materials

The freshly collected plants were rinsed under running tap water and the leaves were dried under shade for 3 weeks. The dried leaves were pulverized by using mechanical grinder to obtain powder form and then stored in airtight containers at room temperature for further progress.

Methods

A. Estimation of Total Phenols

1gm of sample was grind with the help of mortar and pestle with 10 ml of 80% ethanol, and centrifuged at 10,000 rpm (20 minutes). Supernatant was collected and evaporated to dryness. after dryness residue was taken into a test tube and make up the volume with 5ml distilled water. 1 ml aliquot was pipette out in test tube, and make up the volume up to 3 ml with distilled water. 0.5 ml of double diluted Folin- Ciocalteu reagent was added. After 3 minutes, into each tube 2 ml of 20% Na_2CO_3 solution was added. Mixed thoroughly and tubes was kept in boiling water for 1 minute, then allowed to cool and absorbance was measured at 650 nm against reagent blank. reagent blank was prepared similarly without the extract.

Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol (100µg/ml). [25].

B. Estimation of ortho-dihydric phenols

1gm of sample was grind with the help of mortar and pestle with 10 ml of 80% ethanol. And centrifuged at 10,000 rpm (20 minutes). Supernatant was collected and evaporated to dryness. after dryness residue was taken into a test tube and make up the volume with 5ml distilled water. 1ml of extract into the test tube and added 1ml of 0.05N HCl, 1ml of Arnows reagent, 10 ml of distilled water and 2ml of 1N NaOH. And mixed thoroughly (pink color appeared). Reagent blank was prepared similarly without the extract. Absorbance was measured at 515nm against the blank, Presence of ortho-dihydric phenols in the samples was calculated by using standard curve prepared from working standard catechol solution at different concentrations (0.1 to 1ml). [25].

C. Estimation of Bound Phenols

0.1g sample was grind with 5ml of SDS solution. Centrifuged at 2,000g (5min) and supernatant was discarded. washed the residue successively once with 5ml of SDS solution, two times with water, twice with 5ml of ethanol and twice with 10ml of diethyl ether. After each washing centrifuged and supernatant was discarded. Residue was allowed to dry and suspended in 3ml of 0.5M NaOH. Then kept overnight at room temperature. Next day centrifuged and saved the supernatant. Supernatant was diluted 1:10 with 0.5M NaOH. Absorbance was measured at 290nm against the blank prepared similarly without the extract. Presence of bound phenols in the sample was calculated by using standard curve prepared from working standard catechol solution at different concentrations (0.1 to 1ml). [25]

D. Estimation of Tannins

Vanillin hydrochloride method was used to estimate tannin. 0.1 gm of sample was mixed in 5ml methanol after 20-28 hrs. centrifuged and supernatant was collected. 1ml of supernatant was pipette out into test tube and quickly added 5ml of vanillin hydrochloride reagent and mixed. After 20 min absorbance was read at 500nm. A reagent blank was prepared with vanillin hydrochloride reagent alone. A standard graph was prepared from working standard (100µg/ml) of catechin and amount of tannins was calculated. [25].

E. Estimation of Quinones

1gm sample was grind with the help of mortar and pestle by using chilled phosphate buffer (5ml for each gm of tissue). The supernatant was collected after centrifugation for 30 minutes this was used as enzyme extract. 3ml of buffer, 3ml of standard catechol and 1.5 ml of enzyme extract was pipetted in a test tube. It was shaken gently and then placed in water bath for incubation. 4ml of TCA (Trichloro acetic acid) reagent (without ascorbic acid) to one and 4ml of TCA reagent (with ascorbic acid) was added. Precipitate was filtered. Absorbance was measured at 400 nm against a reagent bank lacking only extract. Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of working standard catechol. [25].

F. Estimation of Total Flavonoid content

The Aluminum chloride method was used to determine the total flavonoid content. In brief, 1gm sample was grind with the help of mortar and pestle with 10ml methanol. Centrifuged and supernatant was collected. 0.5ml of supernatant was pipette out into test tube. 0.5 mL of each extract (1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water was added. The reaction was left for completion for 30 min. and absorbance was measured at 415 nm against a methanolic blank (80% methanol). Standard curve was prepared using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 ml) of catechol [26].

III. RESULTS AND DISCUSSION

In the present investigation estimation of secondary metabolites such as total phenol, ortho-dihydric phenols, Bound Phenols, Tannins, Quinones and Total Flavonoid were performed by using spectrophotometer.

Table no 1. Content of secondary metabolites in the leaves of *Euphorbia hirta* L. and *Acalypha indica* L.

Sr no	Secondary metabolites	Wt.of material	*Acalypha indica* L.	*Euphorbia hirta* L.
			µg/g	µg/g
1	Total phenol	1g	140	20
2	ortho-dihydric phenols	1g	15	15
3	Bound phenols	0.1g	300	140
4	Tannins	0.1g	50	45
5	Quinones	1g	14,400	2,780
6	Total Flavonoid	1g	740	580

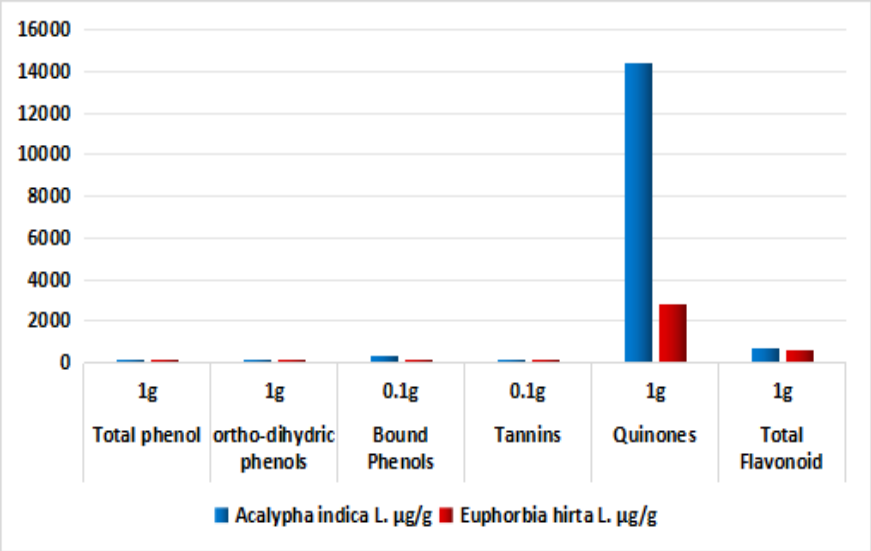

Fig 3. Content of secondary metabolites in the leaves of *Euphorbia hirta* L. and *Acalypha indica* L.

Total phenol, Ortho-dihydric phenols, Bound Phenols, Tannins, Quinones and Total Flavonoid content in the leaves of Euphorbia *hirta* L. and leaves of *Acalypha indica* L. are presented in the table no.1.

Phenolic compounds are the major plant compounds with antioxidant, antimicrobial and antifungal activities and also enhance the flavour and textures of food products [27]. The amount of total phenol, ortho-dihydric phenols, Bound phenols, Tannins, Quinones and Total Flavonoid of the leaves of Euphorbia *hirta* L. were 20µg/g; 15µg/g; 140µg/g, 45µg/g, 2,780µg/g, and 580µg/g. The amount of total phenol, ortho-dihydric phenols, Bound phenols, Tannins, Quinones and Total Flavonoid of the leaves of Acalypha *indica L.* were 140µg/g; 15µg/g; 300µg/g; 50µg/g; 14,400µg/g; 740µg/g.

Phenols and flavonoids, these phytoconstituents are being used in allopathic as well as traditionally as ethnomedicines for antiviral activity such as in COVID-19 treatment [28]. Phenolics generally act as hydrogen donors, reducing agents, singlet oxygen quenchers and metal chelators [29]. Phenolic rich food in our diet is good way to provide antioxidants to the body and protect it from harmful effects of free radicals [30]. quinones are large class of antitumor quinones that have been approved for clinical use against various cancers [31]. According to certain reports, flavonoids have anticancer properties because they inhibit protein kinases, which control cellular processes [32]. As per the findings of Ullah and coworkers (2020), medications containing flavonoids (FMD) exhibit superior efficacy against the lung cancer cell lines A549 and L929 [33].

This flavonoid is used to treat a number of illnesses, including asthma, inflammatory bowel diseases, liver and kidney diseases, carcinogenesis and cardiovascular diseases also demonstrates antioxidant and anti-inflammatory properties [34]. Numerous flavonoids have anti-diabetic properties through boosting insulin secretion, lowering insulin resistance, improving hyperglycemia and enhancing skeletal muscle absorption of glucose in a mouse model [35].

IV. CONCLUSION:

In the present investigation it was observed that leaves of *Acalypha indica* L. contain higher amount of Total phenol, Ortho-dihydric phenols, Bound phenols, Tannins, Quinones and Total Flavonoid as compaired to the *Euphorbia hirta* L. The presence of phenols, flavonoids, tannins and quinone in both plants exhibit the antioxidant, anticancer,antiviralactivity, antitumar, anti-inflamatory, anti-diabetic properties.

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