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PREPARATIVE ISOLATION OF FLAVONOIDS FROM *WATTAKAKA VOLUBILIS* (LINN.F.) LEAF EXTRACTS AND ITS ROLE AS ANTIMITOTIC AND ANTIPROLIFERATING AGENT

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ABSTRACT

A large number of medicinal plants have therapeutic potentials. In this study polyphenolic extracts from leaves of *Wattakaka volubilis* have been applied for HPLC, which reports the presence of seven flavonoids. Extracts have been tested for antimitotic and antiproliferating activities. *Allium cepa* root tips were been used to evaluate cytotoxicity. Polyphenolic and methanolic extracts were toxic on roots number, root length and also reduced the mitotic index. Extracts also showed significant results on antiproliferating activity in yeast model.

KEYWORDS: **Antimitotic, Antiproliferating, Polyphenols, Flavonoids, *Wattakaka volubilis.***

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1. INTRODUCTION

The medicinal action of plants are unique to particular plant species or groups and are consistent with this concept as the combination of secondary compounds. These compounds include alkaloids, flavonoids, steroids, phenolics, terpenes etc. Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds, found in plant origin [1]. Flavonoids are widely distributed in all plant parts; they are usually subdivided according to their substituents into flavanols (Kaempferol, Quercetin), anthocyanins, flavones etc. These flavonoids display a remarkable array of biochemical and pharmacological action such as anti-inflammatory, antioxidant and anticancer activities [2]. The continuing problems caused by malignant disease and the failure of conventional chemotherapy to cure advanced invasive carcinoma indicate that new approaches to control the disease are critically needed. The main concept of chemoprevention has become an important and feasible strategy for cancer treatment. The idea is to control the occurrence of cancer by slowing, blocking or reversing the development of the disease through the administration of these naturally occurring compounds [3]. In the present investigation anti mitotic activity of *Wattakaka volubilis* using *Allium cepa* root meristamatic cells has been determined [4]. The yeast model test using *Saccharocymes cerevisiae* also implemented for anticancer studies [5]. This is also called an anti-proliferate activity. *Wattakaka volubilis* (Linn.f.) Stapf which is also known as *Dregea volubilis* is a tall, woody climber commonly found throughout the hotter parts of India. Phytochemical screening of *Wattakaka volubilis* showed presence of alkaloids, anthocyanins, anthracene glycosides, catecholic compounds, coumarins, flavonoids, saponins, triterpenoid glycosides and drevogenins from leaves and steam. *Wattakaka volubilis* is reported to exhibit anti-inflammatory, hepatotoxicity, antioxidant, immune-modulatory activities [6]. It has also been reported that drevogenins isolated from *Wattakaka volubilis* showed neuropharmacological properties [7]. Literature also reveals information on medicinal values of different parts of this plant. The present study deals with the isolation of flavonoids and aimed at establishing the anticancer potential of this plant.

2. MATERIALS AND METHODS

Extraction of plant material

The fresh leaves of *Wattakaka volubilis* (Linn.f.) Stapf were collected during month of August from Khanapur Forest, Bidar. The plant was identified by, Department of Botany, Gulbarga University, Gulbarga, (HGUG No. 83). The shade dried leaves of *Wattakaka volubilis* (Linn.f.) Stapf were powdered to 22 mesh size using the electric blender and then subjected to successive cold extraction with petroleum ether (40^0-60^0c), chloroform, methanol and distilled water. The extracts thus obtained were further evaporated to dryness under vacuum and refrigerated for future use.

Extraction of polyphenols

Finely powdered leaf sample (500 gms) was mixed with 70% ethanol and kept at room temperature for 5 days. After 5 days solution was filtered andsolvent was evaporated. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/ 1ml). Extraction of polyphenols was carried out for 36 hours at room temperature then the aqueous layer was discarded; combined ethyl acetate layer was concentrated which contained polyphenols [8].

HPLC analysis of polyphenols

The HPLC analysis was performed with a reversed-phase HL-459 C 18 column (200 mm x 4.6 mm i.d., 10µm) at room temperature. The mobile phase was acetonitrile-water, the

effluent was monitored at 254 nm and the flow rate was at 1.0 ml/min[9]

Antimitotic activity

Allium cepa has been used for evaluating cytotoxic properties since the early 1920's [10]. This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth inhibition of the roots of onion bulbs. It has been reported that the results from *Allium* test fit in well in a test battery composed of prokaryotes and /or other eukaryotes. Small onion bulbs are carefully unscaled and cultivated on top of test tubes filled with the methanolic and polyphenolic solutions of known amount of extract. Water was used as a control. The test tubes were kept in an incubator at 24±20^{0}C and the test samples were changed daily. After 5 days the roots were counted and their lengths were measured for each onion. When the newly emerged roots measured 2.0 – 3.0 cm, they were fixed. The fixative solution was glacial acetic acid/absolute alcohol (1/3 v/v). The root tips were kept in aceto-alcohol solution for 24 h. After fixation, the slides were prepared for examination or the roots were transferred to 70% ethyl alcohols and stored in a refrigerator. For examination, the root tips were put into a watch glass to which 9 drops of aceto-orcein and 1 drop of 1 M HCl were added and warmed over a flame of spirit lamp for 2-3 min [11]. These tips were kept at room temperature for 15-30 min. After removing the root caps from well-stained root tips, 1 mm of the mitotic zones were immersed in a drop of 45% acetic-acid on a clean slide and squashed under a cover glass. In order to spread the cells evenly on the surface of the slide, squashing was accomplished with a bouncing action by striking the cover glass with a match stick. Mitotic index (MI) was expressed in terms of divided cells/total cells.

Antiproliferating activity

Preparation of yeast inoculums

About 5gms of commerciality available yeast inoculated in the conical flask containing 100 ml sterilized nutrient broth. This conical flask was incubated at 37^{0}C for 24 hrs. This was referred as seeded broth. 1ml of seeded broth was taken and diluted with sterilized distilled water up to 10 ml which contained about 25.4 x 104 cells.

Preparation of extract solution

Methanolic and polyphenolic extracts was dissolved in sterile water were referred as stock solution (100 mg/ml). From stock solutions of each extract of 3 different concentrations were prepared (50, 100, 150 µg/ml) [12].

Cell Viability count

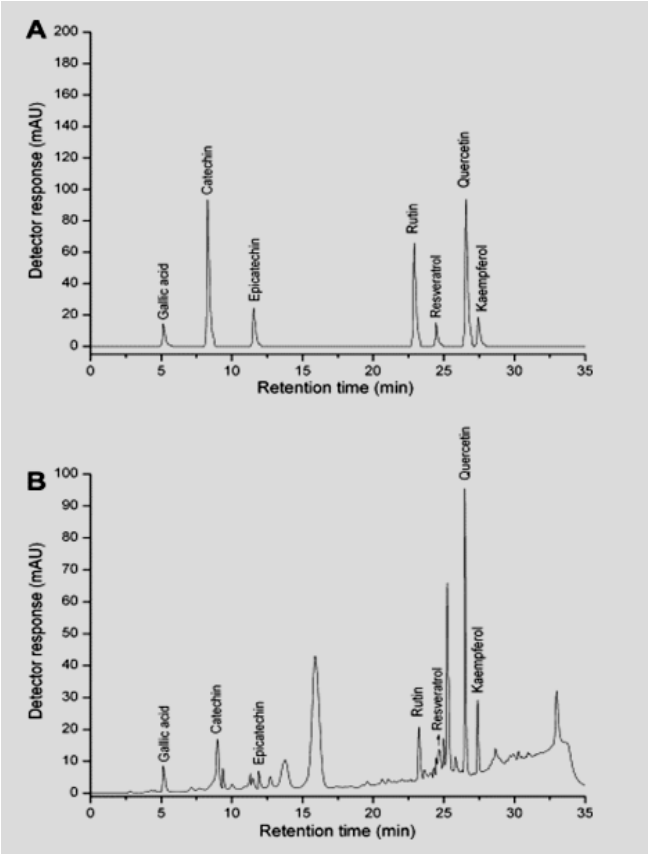
To each 1ml of the extract dilutions, 2.5ml potato dextrose broth and 0.5 ml of yeast inoculums were added. It was then incubated for 24 hours at 37^{o}C, while one was kept as a control. This cell suspension was mixed with 0.1% methylene blue and examined by low-power microscopy. The numbers of viable cells (those which do not take stain and look transparent with, oval shape while dead cells get stained and look blue) were counted in 16 chambers of hemocytometer and the mean was calculated. First of all viable cell count were determined for blanks i.e. water and ethanol. Then viable cell count was determined after treatment with different concentrations of methanol and polyphenolic extracts [12].

3. RESULTS AND DISCUSSION

HPLC analysis of polyphenols

The polyphenolic extract of *Wattakaka volubilis* analyzed by HPLC showed the presence of seven flavonoids, which were run against seven standards such as Kaempferol, gallic acid, rutin, quercetin, catechin, epicatechin and resorcinol as shown in Fig-1.

Figure 1
HPLC analysis of polyphenols of Wattakaka volubilis (Linn.f.) leaf extracts



Antimitotic activity and Antiproliferating activity

The root length and number for control for each extract are given in Table 1. *Wattakaka volubilis* extracts reduced significantly root number and root length when compared with control (tap water). Polyphenolic extract was more effective on root length and number when compared with methanolic extract. This results shows that the extracts from *Wattakaka volubilis* have inhibitory effects on root growth and length in *Allium cepa.* In conformity with animal and human cell cytotoxicity [13, 14]. It was found that *Wattakaka volubilis* extracts have cytotoxic properties also in plant test systems. In Table-2 the mitotic indexes are given for control and for each extract. It is evident that both the extracts reduced the mitotic index significantly. The reduction in number of dividing cells in the root meristem shows the antimitotic effects of the bioactives that are present in *Wattakaka volubilis* extracts. Polyphenolic extract was more effective in reducing the mitotic index when compared with the methanolic extract. *Wattakaka volubilis extracts* contains antimitotic constituents that can stop the mitosis in anywhere of the cell cycle. Furthermore these constituents probably affect the cytoskeleton or tubulin polymerization or degradation.

The antiproliferative assay using the *Saccharomyces cerevisiae* yeast model (Table-3) also showed that the plant is good inhibitor of yeast cell growth. This value varied dose dependently with increasing concentration of the extracts and the IC_{50} were estimated at 47.08 and 38.25 mg/ml respectively (Table- 4). This study has shown that the extracts contain useful anticancer constituents.

In the present study we showed that the methanolic and polyphenolic extracts of *Wattakaka volubilis* leaf extract contain seven flavonoids which are determined by HPLC results, as well as the extracts proving anticancer activity by showing significant results

on antimitotic and antiproliferating activities. Flavonoids display a remarkable spectrum of biological activity that is dysregulated during cancer development [15]. They may therefore have beneficial health effects and can be considered possible chemopreventive or therapeutic agents against cancer [16]. The antimitotic activity using *Allium cepa* root meristamatic cells have been used extensively in the screening of drugs with antimitotic activity. This division is similar to human normal and cancer cell division. Hence, these meristamatic cells can be used for screening of drugs with potential human anti cancer activity [4].

Another model test system for anticancer study uses yeast *Saccharomyces cerevisiae* (Yeast model), this is also called as anti-proliferate activity [5]. The high degree of conservation in terms of sequence similarity and function has made this organism useful in elucidating biological pathways, both yeast and human. Among these are pathways responsible for DNA damage repair and cell cycle control. Therefore yeast model used as a model system for anti-cancer drug discovery. There are several possible explanations for this pattern of activity, as HPLC results depicts the presence of seven flavonoids very particularly each of these flavonoids shows remarkable role in the mechanism of anticancer. The degree of oxidative stress in a cell reflects a balance between the rate of ROS production and the activity of scavenging systems that detoxify them [17]. Increased basal oxidative stress in transformed cells makes them highly dependent on their antioxidant systems to counteract the damaging effect of ROS and this makes them susceptible to further oxidative insults. Diverse chemotherapeutic agents can induce apoptosis in cancer cell through increased ROS generation [18].

Kaempferol is known to induce apoptosis in numerous cancer cells. Our results show that kaempferol induces apoptosis or antimitotic activity by increased ROS generation. Because ROS toxicity induced by certain chemotherapeutic agents can be an effective means of eradicating malignant cells, it is useful to consider effective ways of achieving significant synergy through combination of agents with similar ability to alter redox conditions. Doxorubicin, often included in chemotherapy regimens, has several mechanisms of action, one of which is the formation of free radicals leading to oxidative stress [19]. Kaempferol potentiates the toxic effect of doxorubicin by amplifying ROS generation. The ability of kaempferol to enhance intracellular accumulation of doxorubicin by reducing its efflux agrees with previous studies that flavonoids reverse multidrug resistance involved in regulating the efflux of chemotherapeutic drugs from cells [20].

HPLC showed the presence of quercetin in *Wattakaka volubilis* extracts. Quercetin binds to excess iron in body, removes it from tissues, and prevents its absorption. This process is called chelation. This is critical as iron can be a key ingredient in cancer cell growth. Quercetin has the ability to steal the iron from cancer cells which can stop their growth and induce cell death [21]. Gallic acid as flavonoid also shows anticancer activity. The inhibitory effect of gallic acid on carcinogenesis was mediated through the regulation of multiple signaling pathways. Our results suggest that gallic acid activated cell death extrinsically along with mitochondrial intrinsic pathways and, thus, executed cell death. Gallic acid is known to induce cell death or cell cycle arrest in a variety of cancer cells in apparently different manner [22]. Catechin has anticarcinogenic and antioxidant activity and may prevent oxidative damage in many organs, including heart, kidney, lungs and spleen. Catechins have been found effective in the treatment cancer [23]. Evidence suggests that polyphenolic compounds are able to traverse cell membranes and may enter the cytoplasmic or nuclear space and functions as anticarcinogenic agents [24].

Table 1
The average root lengths and numbers in control and in extracts after 6 days.

Extract	Average root numbers (±SD)	Average root lengths (cm) (±SD)
Control	32±3	6.3±2.5
Methanolic extract	20±4*	3.0±3.0*
Polyphenolic extract	15±3**	1.7±1.5**

Table 2
The dividing and total cells that counted in microscopic observations and mitotic index (MI) in control and in decoction

Extract	Total cells	Dividing cells	MI
Control	10000	1620	16%
Methanolic extract	10000	540	6%
Polyphenolic extract	10000	220	2.8%

Table 3
Result of Antiproliferative assay of extracts of Wattakaka volubilis leaves using the Saccharomyces cerevisiae yeast model assay

Name of extracts	Concentration in (mg/ml) and no. of viable cells per ml		
	10	50	100
Methanolic extract	190000	110000	57000
Polyphenolic extract	140000	90000	48000

Table 4
Result of % Inhibition of viable cell of extracts of Wattakaka volubilis leaves using the Saccharomyces cerevisiae yeast model assay

Name of extracts	Concentration in (mg/ml) and % inhibition of viable cells			IC_{50}
	10	50	100	
Methanolic extract	30.15%	58.21%	79.70%	47.08
Polyphenolic extract	40.90%	60.15%	78.57%	38.25

CONCLUSION

This study indicates that the polyphenolic extract of *Wattakaka volubilis* reports the presence of seven flavonoids and extracts obtained from the leaves of *Wattakaka volubilis* (Linn.f.) Stapf have significant anti mitotic and anti proliferating activity.

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