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Full Length Research Paper

Analyses of the methanolic extract of the leaves of *Rhinacanthus nasutus*

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Medicinal plants are sources of important therapeutic aids for alleviating human ailments. Medicinal herbs are known to contain a variety of antioxidants. Herbal medicine is the use of medicinal plants for the prevention and treatment of diseases, it ranges from traditional and popular medicines of every country to the use of standardized herbal extracts. In tune with this effort, the objective set for the present study is to identify the phytochemical constituents of the leaves of *Rhinacanthus nasutus* in order to understand the nature of the principle component responsible for its medicinal property. A preliminary absorbance survey scan of the methanolic extract of *R. nasutus* evidenced the presence of multiple components in the extract. Two peaks observed in the HPLC spectrum showed the presence of two compounds in the extract. GCMS profile revealed that the active components present in the leaf extract might be alkaloids or polyphenols. The results of IR spectrum revealed that band 1 possess compounds of polyphenolic nature and band 2 possesses compounds that are having a hydroxyl and a carbonyl groups. Some more studies need to be conducted to confirm the presence of bioactive component responsible for its therapeutic value.

Key words: *Rhinacanthus nasutus*, alkaloids, polyphenols, antioxidants, HPLC, GC-MS, IR spectrum.

INTRODUCTION

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. Medicinal plants are considered to be an important source of antioxidant compounds and the therapeutic benefit of many medicinal plants often attributes to their antioxidant properties (Hasan et al., 2007). Antioxidant potential is the sum of a large number of interrelated and interdependent antioxidant systems. Antioxidants are physiological substances that are derived from both endogenous and exogenous sources and that act against oxidative stress (Rai and Phadke, 2006). The balance between antioxidants and oxidation is believed to be a critical concept of maintaining a healthy biological system. Due to the deletion of many bioactive compounds in food with possible antioxidant activity, there has been increased interest in the relationship between antioxidants and diseases (Tarhan et al., 2007).

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. A majority of the antioxidant activity is attributed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes,

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Alzheimer's disease and cancer (Khalaf et al., 2007).

Cancer chemoprevention by phytochemicals may be one of the most feasible approaches for cancer control. Phytochemicals obtained from vegetables, fruits, spices, teas, herbs and medicinal plants, such as alkaloids, terpenoids and other phenolic compounds, have been proven to suppress experimental carcinogenesis in various organs in pre-clinical models. Recent studies have indicated that mechanisms underlying chemopreventive potential may be a combination of antioxidant, anti-inflammatory, immune-enhancing and hormone modulation effects, with modification of drug metabolizing enzymes, influence on cell cycle and cell differentiation, induction of apoptosis, suppression of proliferation and angiogenesis, playing roles in the initiation and secondary modification stages of neoplastic development (Rabi and Gupta, 2008). Thus, the search for crude drugs of plant origin with antioxidant activity has become a central focus of research. In tune with this effort, the present study centered around analyzing the antioxidant activity of the candidate plant *Rhinacanthus nasutus*.

R. nasutus is commonly called as Nagamalli (Tamil). *R. nasutus*. Kurz (Family *Acanthaceae*) is a valuable plant that is widely distributed and cultivated in South China, Taiwan, India and Thailand. *R. nasutus* is well known as a source of flavonoids, steroids, terpenoids, anthraquinones, lignans and especially naphthoquinone analogues. Various parts of this plant have also been used for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases (Siripong et al., 2006).

R. nasutus is an important medicinal plant, which possesses anticancer, antifungal and antiviral properties (Sudhakar et al., 2006). It is a shrub, about 2 to 3 feet tall (Gotoh et al., 2004). Some of the bioactive components of the plant are known to be naphthoquinones such as rhinacanthins (A-D, G-Q), rhinacanthone and lignan groups (Yamazaki et al., 2006). It is extensively used in traditional medicine, to treat liver disorders, skin diseases, peptic ulcers, helminthiasis, scurvy, inflammation and obesity. The methanolic root extract of *R. nasutus* was studied for its hepatoprotective effect. *R. nasutus* helped to preserve an almost normal structure of the liver, following CCl_4 – induced liver damage, indicating its hepatoprotective effects. *R. nasutus* possesses a significant hepatoprotective activity, comparable to that of silymarin (Sharma, 2003).

There is increased quest to obtain natural antioxidants of broad spectrum action. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically explored for such properties. The aim of this research work is to identify the phytochemical constituents present in the leaves in order to understand the structure of the active component responsible for its therapeutic value.

MATERIALS AND METHODS

The methanolic extract prepared from *R. nasutus* leaves were tested for the presence of various known phytochemicals (Khandelwal et al., 2002).

Preparation of plant extracts

Fresh leaves of *R. nasutus* were collected and 1g of them was homogenized thoroughly in 10 ml of appropriate solvent. The organic extracts were dried at 60°C protected from light. The residue was weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain a final concentration of 20 mg in 5 µl of DMSO. Aqueous extracts were prepared fresh when experiments were performed.

Spectral analysis

A preliminary spectral analysis was done by a survey scan of the methanolic extract of *R. nasutus* in a nanospectrophotometer (Optizen, Korea) after which the samples were taken for HPLC analysis.

HPLC analysis

HPLC analysis was conducted with a Shimadzu chromatograph equipped with photodiode array detector and a 250 mm reverse phase column. Shade dried *R. nasutus* leaves were powdered and dissolved in appropriate volume of HPLC grade methanol solvent and injected into the apparatus. The sample analysis of the powdered leaf sample was performed at room temperature, in the wavelength range of 200 – 400 nm at 1000 psi and the mobile phase used was acetonitrile and water in the ratio of 60:40.

Gas chromatography-MS analysis

The powdered plant material was analyzed using an Agilant-5 gas chromatography-MS spectrometer using a HP-5 column equipped with SEM detector with helium as a carrier gas at a flow rate of 1.5psi. The compounds were identified using the database available in the light of the available literature in the journals and books.

IR spectral analysis

Prior to IR analysis, the methanolic extract of *R. nasutus* was subjected to thin layer chromatography using the solvent system ethyl acetate and ethanol in 1:1 ratio. It gave three spots, the middle spot being more distinct, indicative of a single compound. The third spot corresponds to chlorophyll and was not considered. The first and second spots were then separated using preparative TLC and eluted into methanol, and the IR spectral analysis were carried out with the eluted spots using IR spectrophotomer (Shimadzu).

RESULTS AND DISCUSSION

A preliminary phytochemical screening was conducted to identify the chemical nature of the principle component. Qualitative analysis of the methanolic extract revealed the presence of alkaloids, phenolics and flavonoids in the

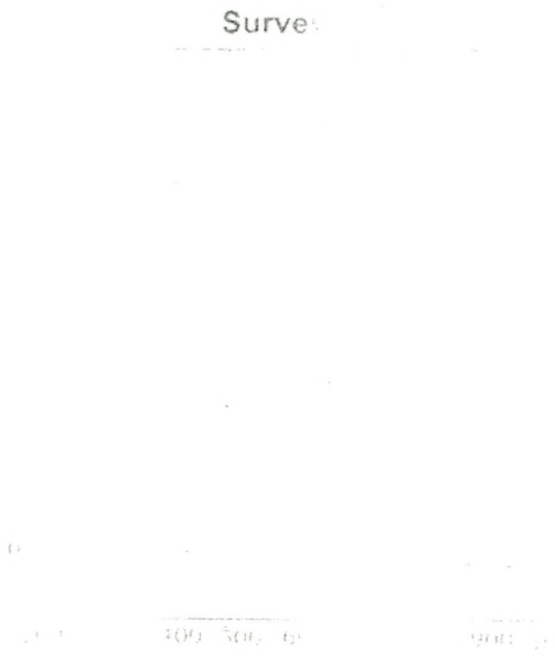

Figure 1. Absorbance spectrum.

leaf extracts of *R. nasutus*. An absorbance survey scan of the methanolic extract of *R. nasutus* leaves in the wavelength ranging from 190 - 1100nm (Figure 1) revealed the presence of multiple components in the extract as evident by the presence of different peaks in the survey scan. In order to confirm the results obtained after qualitative analysis, spectral analysis using HPLC, GC-MS and IR spectral analyses were carried out.

HPLC analysis

Two peaks (a major and a minor one) were observed in the spectrum (Figure 2) showing the presence of two principle components in the leaf extract of *R. nasutus* that might be responsible for its medicinal property. Further spectral studies like GC-MS and Infra Red spectral analysis were followed to identify the nature of these active components.

Gas chromatography-MS analysis

The GC-MS analysis of the leaf sample of *R. nasutus* was carried out to identify the nature of the components present. The GC-MS output also showed the presence of two major components at retention times 4.14 and 7.8 (Figure 3). The respective fragmentation patterns of the components are shown in Figures 3a and 3b. The mass spectrum of the compound with retention time 4.14 gave three major peaks (m/z) at 129.7826, 101.8992, and 76.0586. The element combination for the molecular ion peak at 129.7826 shows the probability for $C_8H_{19}N$, $C7H_5NO$, $C_6H_{11}NO_2$, C_9H_7N and $C_5H_7NO_3$. The above molecular formula indicates the possibility of an aromatic Nitrogen containing compound that is an alkaloid. Also the fragmentation pattern shows loss of CO, N_2, CH_2N or C_2H_4 (129-101-loss of 28) indicating the presence of Nitrogen again. Therefore, it is perceivable that the compound at retention time 4.14 is possibly be an alkaloid. The mass spectrum of the compound with retention time 7.8 gave eight major peaks (m/z) at 221, 203, 165, 147, 119, 102, 91 and 75. The fragmentation pattern of 119, 102, 91 and 75 is shown characteristically by aromatic alcohols or polyols. Also, the loss of m/z -18 (loss of H_2O) from molecular ion peak (221 – 203 - 18) is seen, which is again characteristic of alcohols or phenols. Therefore, it may be concluded that the compound with retention time 7.8 may be a polyphenolic compound.

IR analysis

The results of IR spectrum of band 1 (Figure 4a) obtained from TLC showed major peaks at 3400 cm^{-1} (broad and strong indication of OH), 2923 cm^{-1}(indicative of -C-H stretching), 1741 cm^{-1}(indicative of the presence of lactone ring) and,1166 cm^{-1}(indicative of C-O stretching). Hence, it may be concluded that band 1 contains a compound of polyphenolic nature. The presence of lactone rings indicates the presence of flavonoids or coumarins. Band 2 was a single spot indicative of one compound. The IR spectrum of band 2 (Figure 4b) obtained from TLC showed a broad peak at 3440 cm^{-1} indicative of hydroxyl group (-OH), a sharp peak at 1654 cm^{-1} indicative of carbonyl group. Therefore, this band must possess compounds that are having a hydroxyl and carbonyl group. Thus, the results and observations presented clearly demonstrate the presence of compounds such as alkaloids and polyphenols in the leaves of *R. nasutus*.

Conclusion

Earlier studies carried out using the leaves of *R. nasutus* revealed that the leaves are found to be a potential source of antioxidants (Nirmaladevi and Padma, 2008). The present study aimed at identifying the nature of the components responsible for their antioxidant activity. Phytochemicals and plant extracts, present in fruits, vegetables, plants, herbs and beverages, have been shown to have antioxidant potential that may modulate the etiology of certain chronic diseases (Carpenter et al., 2006). Thirupathi et al. (2008) have reported a wide range of compounds like pyrrolizidine alkaloids, cardoquinones, tannins, phenyl propanoid derivatives and triterpenes to be bioactive. Flavonoids,

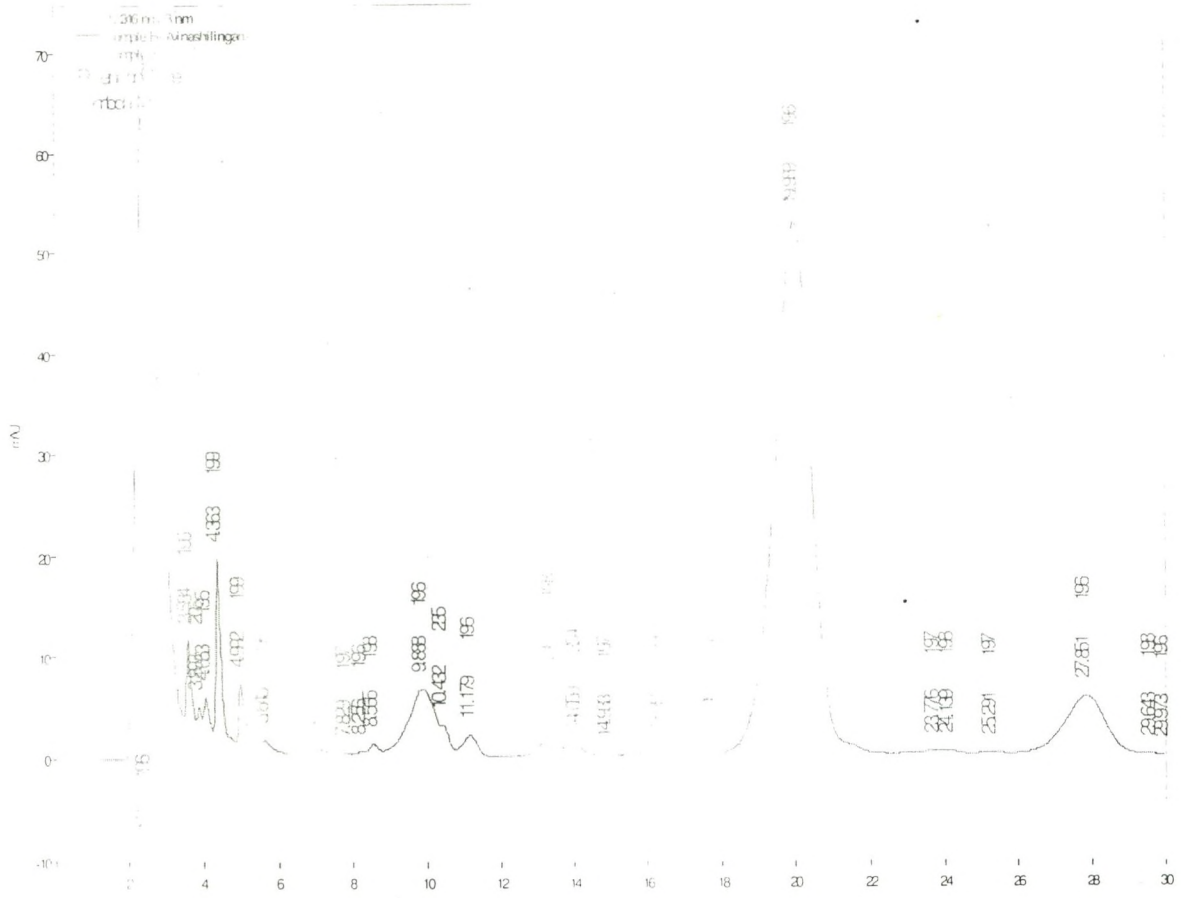

Figure 2. HPLC profile.

Figure 3. Gas chromatography -MS profile.

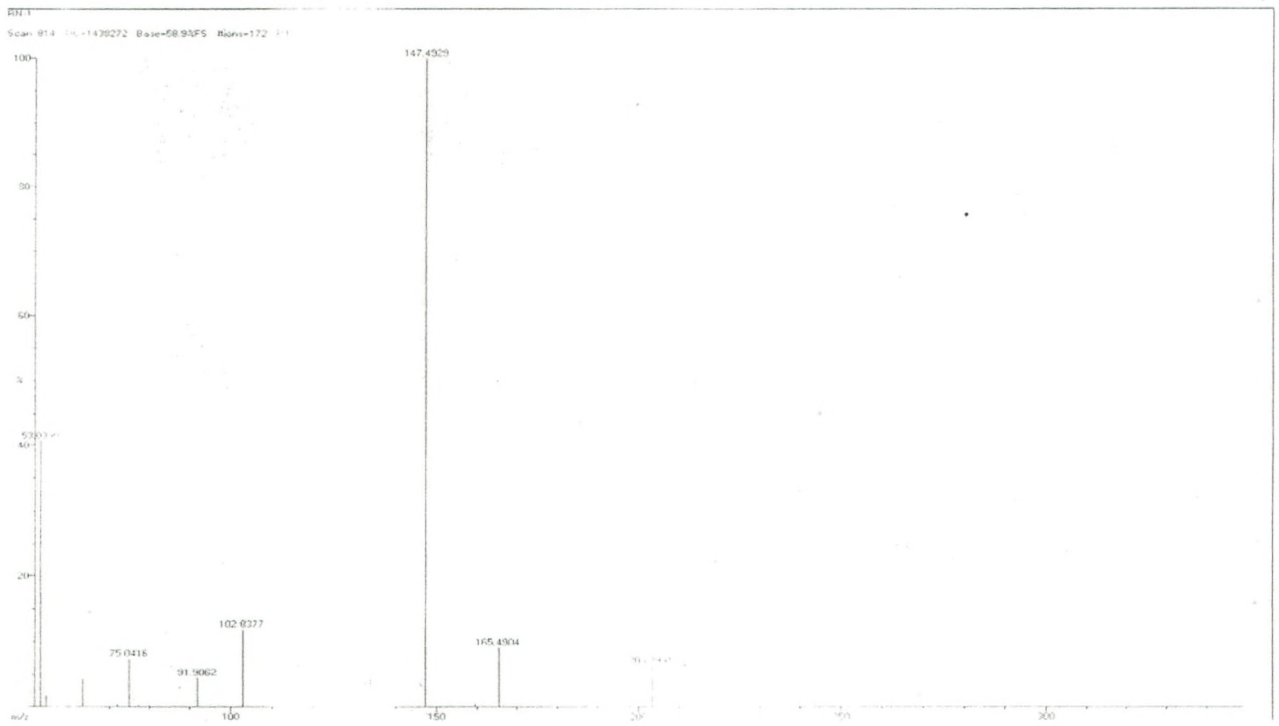

Figure 3b. Peak Fragmentation of gas chromatography -MS spectrum (7.8).

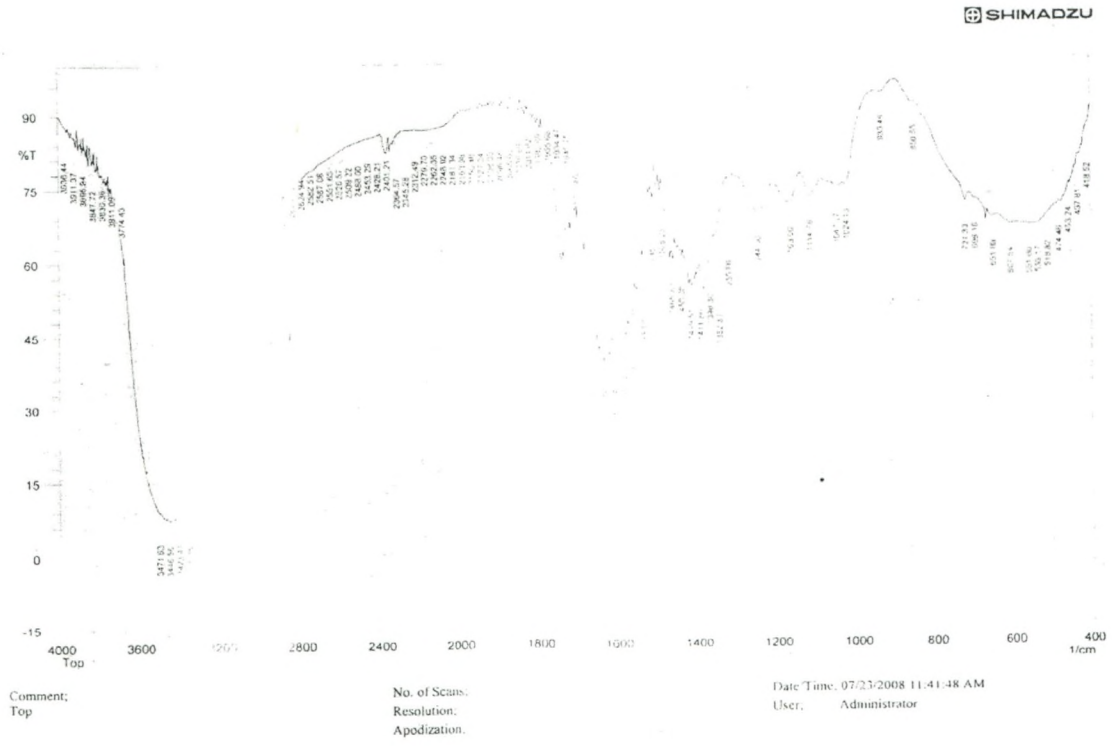

Figure 4a. IR spectrum of band 1.

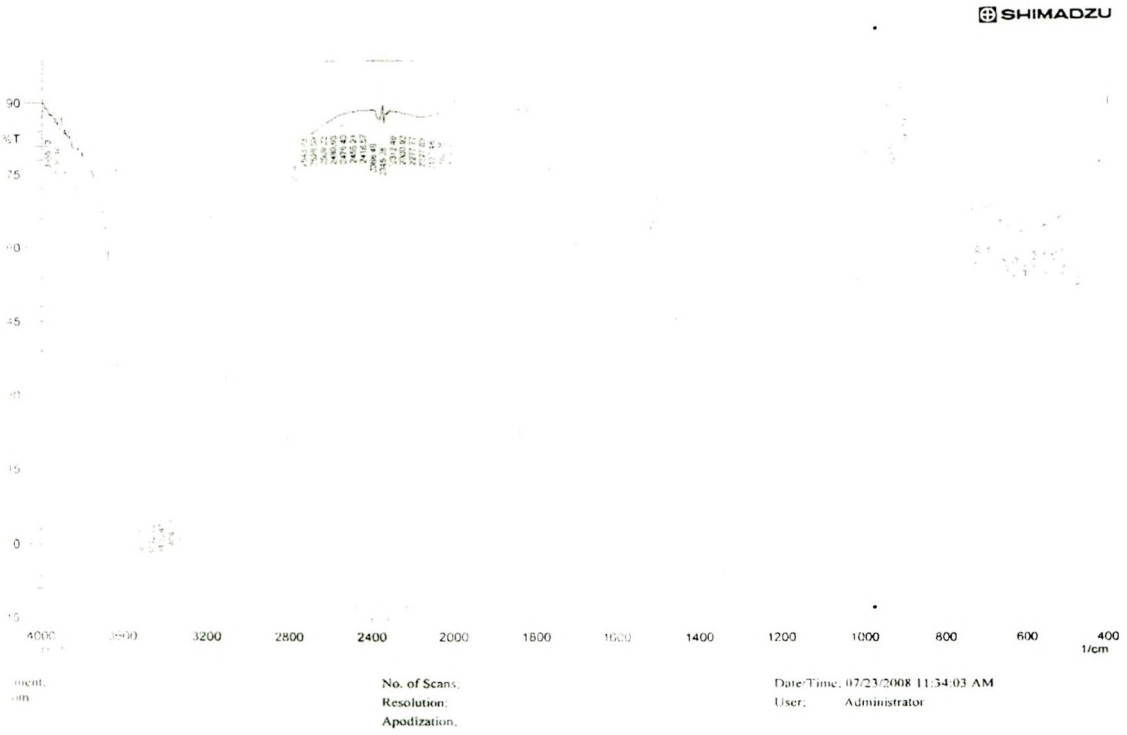

Fig. 1b. IR spectrum of band 2.

anthrac s, terpenes, sterols and other compo olate n *R. nasutus* have been shown to have p *n vitro* antifungal activity (Send et al., 1996). Rhina s-C, - and-Q, three main naphthoquinone esters. d fr e roots of *R. nasutus* were found to ind tox human cervical carcinoma HeLa S3 ce ag , 2006). The ethanolic extracts of seven pecie und in Zimbabwe were found to pose olic es well as antioxidant activity (Mur t al.

S lies shown that the higher antioxidant acti ated medicinal plants is attributed to the pounds (Manjunatha, 2006). The ana micals in the methanolic extracts rev d alkaloids to be the active co robably the major players in the an evoked by the plant. Further st e conducted to understand the str compounds predicted from the ph

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