Journal of Advanced Zoology
ISSN: 0253-7214
Volume 43 Issue S-2 Year 2022 Page 01-08

Isolation And Quantification Of Flavonoids From Selected Medicinal Plants

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Abstract

Derris elliptica belongs to leguminous family from Southeast Asia including Pacific islands. *Ailanthus excelsa* Roxb. (Simaroubaceae) is identified by its ***Mahanimba*** as it has similar features *Azadirachita indica* and ***Maharukha*** as it appears big size. *Ailanthus* word is from ailanto which meaning tree of heaven and also by other names in Indian local languages. Various parts of the plant are used for treatment of the diseases as well as for different healing activities of human beings as well as animals across the globe especially in India and China. There are several phytochemical present in plants, viz. flavonoids,tannins phytosterols, alkaloids and triterpenes, etc. Flavonoids are an unusually large group of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom. In the present study, focus has been made to identify the flavonoid in different samples of *Derris elliptica* L.and *Ailanthus excelsa* Roxb. by TLC and IR. In *D. elliptica*, free form of total flavonoid contents were maximal in leaf (0.76 mg/gdw) while lower in bark (0.61 mg/gdw). Free form of quercetin was found to be maximum in fruit of *P. murex*. Bound form of total flavonid contents were highest in leaves (0.41 mg/gdw) Bound form of flavonoids was lower in quantity as compared to free form . Total flavonoids (free + bound) were highest in leaves (1.38 mg/gdw) while least in bark (0.96 mg/gdw). Free form of all flavonoids occurred highest in leaves (0.64 mg/gdw) while lowest in bark (0.37 mg/gdw) of *Ailanthus excelsa,* whereas bound form of total flavonoids were maximum in leaves (0.36 mg/gdw) while minimum in bark (0.35 mg/gdw). Total flavonoids amount (free + bound) in *A. excelsa* present maximum in leaves (1.00 mg/gdw) and least in bark (0.62 mg/gdw).

Key words: Flavonoids; FT-IR; TLC; Derris elliptica; Ailanthus excelsa

Introduction

Plants are considered as vast reservoirs, as they have potency to accumulate diverse range of natural products termed as "*Bioactive compounds*". The plants possess products were known to ancient era for various remedial efficacies. Reports are available which proves their route of efficacy and accumulation of secondary metabolites and nutraceuticals present in them. The broad range of therapeutic application of plants for a various applications thus assist to produce new bioactives thus having chance for syngertical chemistry on the new molecules .

Flavonoids, are group of bioactive compounds , have gained lot of attention. Further due to their diverse nature, they also possess diverse therapeutic applications thus attracting scientific communities. Flavonoids are natural products that possess a benzo--pyrone moieties in their morphology , made by various biochemical routes like phenylpropanoid, the shikimate and the flavonoid pathway (Dias et al., 2021; Nabavi et al., 2020; Rehan et al., 2021; Tariq et al., 2023). Their biological features, therapeutic application, and bioavailability are strongly connected and estimated based on the chemical moeties. Further they can be divided into 6 major classes: (i) flavanones, (ii) flavones, (iii) isoflavones, (iv) flavonols, (v) flavanols, and (vi) anthocyanins . Edible items are an essential key reservoirs of flavonoids, present investigations also proves that prolaryotes , like fungi and bacteria, produce flavonoids from floras (Okoye et al., 2023;. Ververidis et al., 2007).

Derris elliptica belongs to leguminous family from Southeast Asia including Pacific islands. The roots of *D. elliptica* possess rotenone, a robust insecticide and fish poison present in roots, (Fryer et al., 1923) it was earlier recommended as an natural insecticide to fight against crop pests like peas. However, as there are some cases regarding side effects of rotenone it is not beneficial though it has vast potential . Derris root, when crushed, yields rotenone. Some innate residents of Fiji and New Guinea rehearsal fishing in which they crush the roots and mix them with the water. The stunned fish drift to the surface where they can be easily stretched. Inspite of all this Derris is used as a food plant by *Lepidopteran* larvae including *Batrachedra amydraula.* There are studies on biocompatibilities of this plant. Jessa et al (2015) reported the efficacy of dehydrated extract of *Derris elliptica* stem are responsible for some enzymatic variations in the plasma of *Clarias gariepinus.* However Paul et al (2019) reported study on therapeutic screening of *Derris robusta.*

Ailanthus excelsa Roxb. (Simaroubaceae) is identified by its ***Mahanimba*** as it has similar features *Azadirachita indica* and ***Maharukha*** as it appears big size. *Ailanthus* word is from ailanto which meaning tree of heaven and also by other names in Indian local languages (Database, 2000)

Classical names: Araluka, Aralu, Katvanga, Deerghavrinta, Putiveriksha.

Plant is deciduous tree, 19-26 m including trunk straight, 65 to 85 cm in width ; having taste like aromatic bitter slightly. In Indonesian it is identified by name as *Ailanthus moluccana* (Lavhale and Mishra, 2007). The leaves are recommended as an supplement for *Adhatoda zeylanica*/ (*Adhatoda vasica* Nees.). Stem is excellent replacement for kutaj (*Holarrhena antidysenterica* Wall.). It has been reported as sophisticated as an boulevard tree for its yawning shade and is in conventional application for to fight against erosions. It flourishes best on porous loamy soil. It can be grown from both seeds and have vegetative propagation mode . Its fast progress and unqualified defensive strategies to nibbling attracts it among the soft woods. The leaves can be highly edible and and have nutritional properties for livestock animals and an average tree yields about 500-700 kg of green leaves twice a year. The bark is full of lucidity, yellowish white and perfectly designed for cabinet making (Bhandari and Gupta, 1972).

Ethnopharmacology

In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery, especially when there is a blood in stool (Dash and Padhy, 2006). *Ailanthus excelsa* is a fast growing tree and is extensively cultivated in many parts of India in the vicinity of villages; it is cultivated as an avenue tree for its deep shade and can be used for ant-erosion purposes . The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma (Kirtikar and Basu, 2003). In Africa the plant is used to treat *Ailantic* acid cramps, gonorrhea epilepsy, tape warm infestation and high blood pressure (Sharma, 1996). The root bark is used to cure epilepsy and heart troubles.

The bark is bitter, astringent, anthelmentic, febrifuge, appetizer, bitter tonic, taste bud stimulant. It is useful in and Sonapatha/Shyonak is mentioned as its synonym. In diarrhea, amoebic dysentery, chronic giardiasis, dyspepsia, abdominal spasm anorectal disease, haemorrhoids, fistula, fissures, ulcerative colitis and worm infection. It is also used as blood purifier in skin diseases, typhoid fevers, blood coagulation disorders, gouty arthritis, boils, carbuncle, scadies and allied skin disease, chronic bronchitis, bronchial asthama, pulmonary kochs, bronchiectasis, polyurea, diabetesmelitus, obesity,uterine disorders like dysmenorrhoea and lecorrhoea. The bark and leaves have great repute as postnatal tonic. Leaf juice is administered along with milk for post labour pains.

Pharmacological properties

Antifertility efficacy: The alcoholic extracts of different plant parts at concentration of 255mg/kg b.wt. showed are markable anti scion and early antifertility efficacy in female albino mice(Lavhale and Mishra, 2007).

Anti-amoebic activity:
The polar and non polar extracts (Quassinoid fraction) of stem bark was tested against *Entamoeba histolytica* for its anti-amoebic properties using metronidazole as market drug. The EC value for these extracts was found to be in range of were 150-200 µg mL/g (Yoganandam *et al*., 2009a).

Gastroprotective and antisecretory effects:
This activity was reported by Melanchauski *et al*., 2010.

Antiasthmatic efficacy : Alcoholic extract of stem barks have potent efficacy to combat respiratory disorders thus having this activity in mice (Kumar *et al*., 2010 a,b).

Materials and Methods

Collection of Plant Material
The Plant material of *Derris elliptica* were collected from the outskirts of Jamwaramgarh from Jaipur district. The plant of authenticated from Rajasthan University, Jaipur, India. (Ref. RU/2019/532). The different plant parts (bark and leaves were shade dried initially.
Different Plant parts of ***Ailanthus excels*** (Bark and leaves) were harvested from Jaipur, University of Rajasthan. Further it was authenticated from Rajasthan University, Jaipur, India. (Ref. RU/2019/841). The plant materials were cleaned instantly with running tap water, and moisture was removed in closed room and were made to powder through grinder.

Extraction
Various plant parts of selected plants were dehydrated in shade and grinded , discretely. They were macerated with 80% methanol with heating mantle (Subramanian and Nagarajan, 1969) at time interval of 1 day . Layer which were dissolved in methanol were extracted out , diluted *in vacuo* and water soluble compounds were purified by chronological drawing out with petroleum ether (Fr1), diethyl ether (FrII) and ethyl acetate (FrIII) consecutively . Each process followed thrice for complete isolation , first fraction was redundant in every process as it was heavily interfered with fatty compounds, while 2^{nd} and 3^{rd} fraction were isolated differently there after processed further for analysis.

Fractions of ethyl acetate were acidified by treating with 7% H_2SO_4 (10mL /g sample for 2 h), filtered and remains 3 times treated from ethyl acetate. Complete fractionated layer were pooled discretely, pH was adjusted through distilled water during frequent reactions along with dehydrated *in vacuo.* Both fractions were pooled out in minute quantity and dissolved in ethanol (2-5mL) prior chromatographic analysis.

Qualitative
Thin Layer Chromatography
Slender glass plates (20x20 cm) were entrapped from Silica gel G (250mµ in size). Recently made silica gel coated plates were dehydrated around 37^0C; later on they were heated at 100 ^{0} C for half an hour to trigger and then chilled at 37^0C. The freshly made and activated plates were carried further for experimentation.

Tested sample were analyzed with pure compound as standard. (quercetin, luteolin and kaempferol). Further plates were prepared in capped TLC assembly room inundated having mobile phase (Benzene: Acetic Acid: Water:: 125:72:3; (Wong and Francis, 1968). Saturated plates same dehydrated and observed in UV light by treating with some vapors of NH_3. Each spot was exposed to flask saturated with concentrated NH_4OH (100 mL) for about 10-15 seconds and colored marks parallel to pure standard were screened. The saturated plates sprinkled with 5% $FeCl_3$, 0.1% $AlCl_3$ having traces of alcohol and put in I_2 cavity disjointedly. Compounds were observed as fluorescence, were marked while their resolution factor coinciding each spot was measured. Other saturated solvents like n- butanol, acetic acid, water (4:1:5), tertiary butanol, acetic acid, water (3:1:1) have been screened, however mobile phase saturated with benzene, acetic acid, water (125:72:3) was best.

Preparative thin layer chromatography
This technique discussed above of flavonoid samples were performed in same manner on glass plates have coating of silica gel G (BDH ; 500mµ size) by marking sample along with standard flavonoids (luteolin, kaempferol and quercetin). Further coated silica glass plates were saturated in mobile phase of benzene,

acetic acid, and water (125:72:3), dehydrated and observed in UV light. Bands parallel along pure markers marked , frayed from 200 plates, dissolved in 50% methanol. The isolated components were filtered, dehydrated and once more analyzed on chromatogram in parallel with pure standards to screen their transparency. The separated phase were made to crystallization disjointedly and mp, mmp was calculated . The fractions were carried forward for UV and FT-IR analysis.

Identification

The distinctiveness of extracted compounds were proved by mp, mmp performed in capillaries, IR (Infra-red spectrophotometer; Perkin, Elmer 337, Grating Infra-red spectrophotometer), UV estimation in parallel with along standard markers.

Quantification

The purified samples were determine through their optical density using protocol of Mabry *et al* (1970).

Different dilutions ($1mgL^{-1}$) of kaempferol, luteolin and quercetin were made discretely through mixing pure markers in CH3OH. Various dilutions in concentration of 20µg to 160µg of each extract marked discretely on coated plates with silica gel G. For every dilution of tested samples different plates of pure markers applied and saturated in the similar way as mentioned above. The saturated coated plates were dehydrated and observed in UV light. The colored marks were noted and absorbance was noted down of each and every eluted fraction. Higher grade CH_3OH (5mL) was amalgated in every tube , bewildered vigorously, centrifuged and liquid fractions were pooled discretely. The reaction mixture of purified sample was raised to 10mL by mixing CH_3OH. In every test material , 3mL of 0.1 M $AlCl_3$ reaction mixture mixed repeatedly astonished forcefully and maintained at 37^0 C at time interval of 20 min. Five repetitions occured in same manner and its absorbance were determined at 426nm for kaempferol and luteolin while for quercetin at 440nm in contrast to blank (10ml of higher grade CH_3OH and 3mL of 0.1 M $AlCl_3$). Regression peaks were plotted among dilutions and in response to mean absorbance of each compound. The standard curve prepared followed Beer's law.

Every test sample obtained above in different fractions (ether and ethyl acetate diluted) was added in 5 mL of CH_3OH and 0.1mL was put glass TLC silica gel entrapped plates in parallel along standards, discretely. Plates were saturated in same manner and the spots matching in pure standard on every coated plate in UV light. Each band was eluted along with silica , dissolved in CH_3OH and test sample carried out in the similar manner as earlier done . The absorbance of every dilution noted and dilution of tested sample was prepared through the standard peaks of pure flavonoids markers. Final dilutions thus obtained were quantified as mg/g dry weight.

Results and Discussion

3 different spots of flavonoids appeared in different plant parts after TLC when drizzled with 5% $FeCl_3$. Resolution factor of these bands was found to be similar when compared with pure authentic markers and characterized as kaempferol, quercetin and luteolin. Mobile phase having solvents of Benzene: Acetic Acid: Water (125:72:3) had prominent outcomes bearing these resolution value as viz., kaempferol, 0.86; luteolin, 0.56 and quercetin, 0.78. Different solvents like n-Butanol: Acetic acid: Water (4:1:5) and conc. HCl: Acetic acid : Water (3:30:10) were also examined but 1st solvent was best and the R_f value of kaempferol was found to be 0.83 and 0.55, quercetin having 0.64 and 0.41 and luteolin having 0.83 and 0.77 **(Fig. 1-3)**.
The purified compounds viz., kaempferol quercetin and luteolin were identified and analyzed by coinciding IR peaks and mp (kaempferol, 273-275°C; luteolin 328-330°C; quercetin 311-313°C and UV spectra (kaempferol, 253sh, 266, 394sh, 322sh, 368; quercetin 255, 269sh, 301sh 374; luteolin 242sh, 253, 267, 291sh, 349), which were coinciding with pure compounds.

Quantitative analysis

In *D. elliptica*, free form of total flavonoid contents were maximal in leaf (0.76 mg/gdw) while lower in bark (0.61 mg/gdw). Free form of quercetin was found to be maximum in fruit of *P. murex*. Bound form of total flavonid contents were highest in leaves (0.41 mg/gdw) Bound form of flavonoids was lower in quantity as compared to free form . Total flavonoids (free + bound) were highest in leaves (1.38 mg/gdw) while least in bark (0.96 mg/gdw) (**Fig. 4**).

Free form of all flavonoids occurred highest in leaves (0.64 mg/gdw) while lowest in bark (0.37 mg/gdw) of ***Ailanthus excelsa,*** whereas bound form of total flavonoids were maximum in leaves (0.36 mg/gdw) while minimum in bark (0.35 mg/gdw). Total flavonoids amount (free + bound) in ***A. excelsa*** present maximum in leaves (1.00 mg/gdw) and least in bark (0.62 mg/gdw) (**Fig.5**).
Consumption of natural resources in developed nations has prolonged mainly in the later decade of 20th century. Monographs on specific medicinal plants have been observed in different resources, like European Scientific Cooperative on Phytotherapy (ESCOP, 1999), German Commission E (Blumenthal , 1999). Their monographs, mention the herb by many factors (including synonyms and vernacular names) and plant organ normally consumed , its geographical location , factors to recognize and specify the plant (including macroscopic and microscopic screening and testing their purity and efficacy), the natural products engaged dose level along with method of administration , pharmacological applications , contra-indications and side effects (Ogundele and Das, 2019).

Several species of medicinal plant have exploited at large scale for isolation of novel drugs with tremendous efficacy and reduced toxicity (Srivastava et al., 2007). They are also considered rich deposits of many bioactive compounds which have several pharmacological efficacies (Farnsworth, 1989; ,Eisner, 1990). The natural products in the form of various metabolites have rich deposits of various phytochemicals that could act as precursors for synthesis of novel molecules. Conventional medicine have even better application in current specific medical ailments mode of the emerging nations. The bioactive compounds are beneficial to human body, in contrast to currently recommended chemically based medicines. Therefore crucial point urge to gain highest profit from conventional practices of drugs for assisting ample medical ailments to common community (Ghani, 1990).
There are many causes for quantification of variety of flavonoids in plants from which certain edibles are obtained (Hertog et al., 1992). These kind of investigations will assist scientific community to help scientists, like, with investigations of therapeutic effects of flavonoid ultimately results for better knowledge to determine the proper adequate consumption of flavonoids.
3 different spots of flavonoids appeared in different plant parts after TLC when drilled with 5% $FeCl_3$. Resolution factor of bands was found to be similar when compared with pure authentic markers and characterized as kaempferol, quercetin and luteolin. Mobile phase having solvents of Benzene: Acetic Acid: Water (125:72:3) gave prominent outcomes.The extracted fractions viz., kaempferol quercetin and luteolin has been screened and identified through super imposable IR bands along with mp.
In *D. elliptica*, free form of total flavonoid contents were maximal in leaf and lower in bark. Free form of quercetin was found to be maximum in fruit of *P. murex*. Bound form of total flavonoid contents were found to be maximum in leaves Bound form of flavonoids was lower in quantity as compared to free form . Total flavonoids (free + bound) were highest in leaves and least in bark. Free form of all flavonoids observed maximum in leaves and lowest in bark of ***Ailanthus excelsa,*** whereas bound form of total flavonoids were maximum in leaves and minimum in bark. Total flavonoids amount (free + bound) in ***A. excelsa*** was found to be more in leaves and minimum in bark.

Conflict of Interest
The authors declare that there is no conflict of interest.

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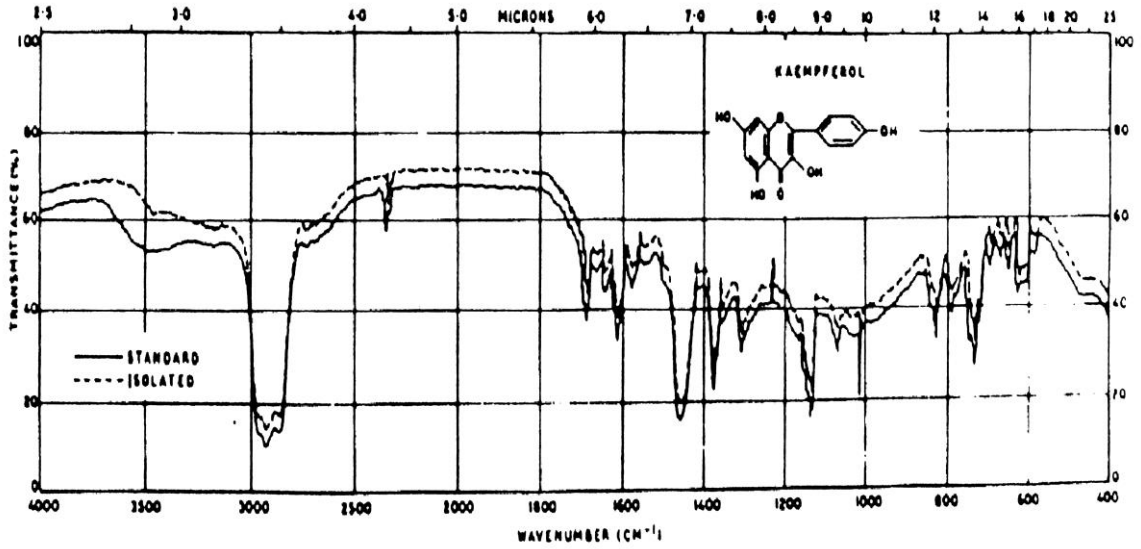

Fig. 1 Infra-red Spectra of Isolated and Standard Kaempferol

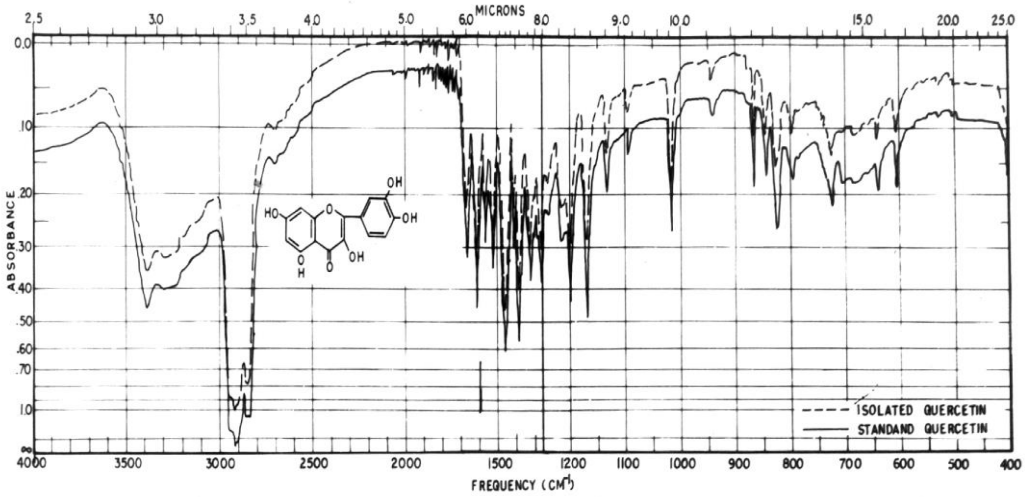

Fig. 2 . Infra-red Spectra of Isolated and Standard Quercetin

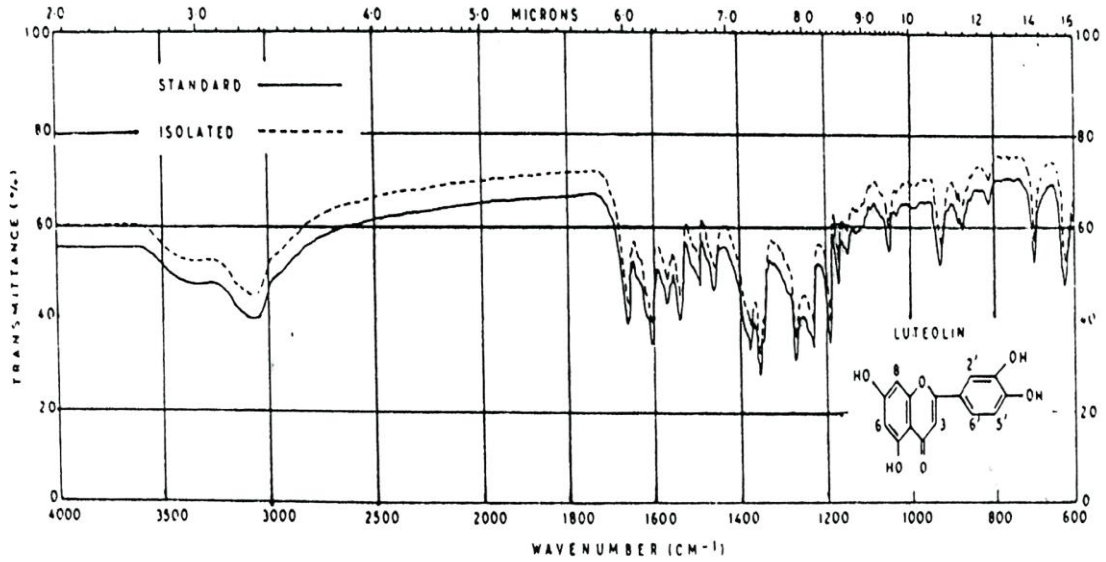

Fig. 3. Infra-red Spectra of Isolated and Standard Luteolin

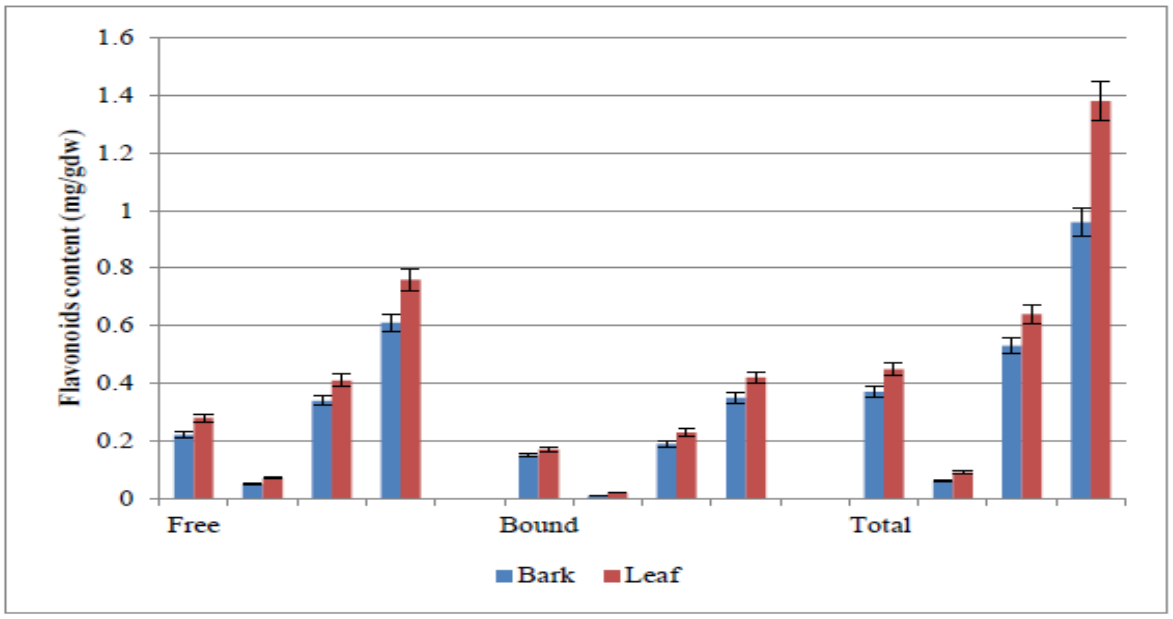

Fig. 4. Flavonoids content (mg/gdw) in various plant parts of *Derris elliptica***.**

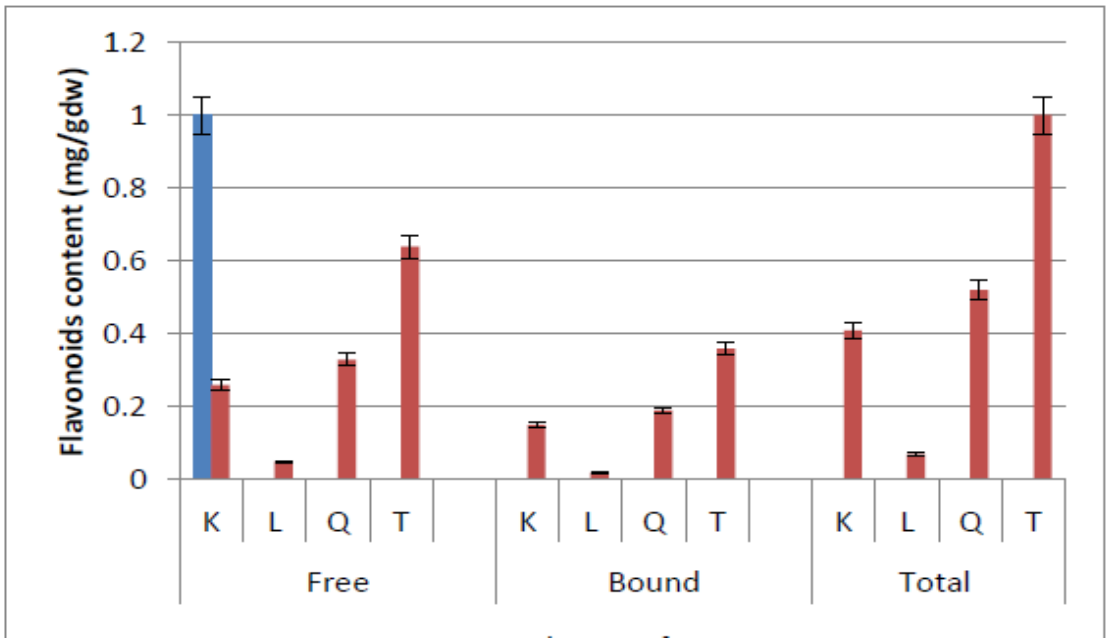

Fig. 5 . Flavonoids content (mg/gdw) in various plant parts of *Ailanthus excelsa*