Estimation of Antioxidants (Flavonoids) in *Ailanthus excelsa* and *Balanites aegyptiaca* and Effect of Growth Regulators and Salts on Antioxidants in vitro

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Abstract: *Ailanthus excelsa* and *Balanites aegyptiaca* are two wild growing common plant species of desert, having medicinal importance. Unorganized tissues of these useful plants were established on MS medium supplemented with 1.0mg/L BAP+1.5mg/L 2,4-D and 1.5mg/L BAP +2.0mg/L 2,4-D respectively (standardized MS medium). Parts of established tissues were transferred to standardized (Sd) MS medium fed with various concentrations (1,2,3 mg/L) of growth regulators (IAA, NAA) and (10,20,30mg/L) salts ( NaCl , KCl ) separately. Tissues at the maximum GI( in all samples) were harvested, dried, powdered and analyzed for estimation of flavonoids. Maximum amount of flavonoids was calculated in callus fed with 1mg/L IAA, NAA and 10 mg/L NaCl and KCl in *A. excelsa* and *B. aegyptiaca*.

Keywords: *Ailanthus excelsa*, *Balanites aegyptiaca*, antioxidants, growth regulators, salts.

1. Introduction

*Ardu* (*Ailanthus excelsa*) belonging to family *Simaraubaceae* is a large deciduous tree having a lot of medicinal uses. It is a native of India and Sri Lanka. The plant is recognized for its varieties of medicinal uses like anticancer, contraceptive, post partum treatment, intestine tape worm, dysentery, epilepsy, heart troubles, asthma etc. The plant also having antibacterial, antimicrobial and antifungal activity. Two new tetracyclic triterpenes (ailexcelone and ailexcelol) were isolated from the heartwood of *Ailanthus excelsa* which are tested for their antifungal activity (*Srinivas et al.*, 2006).

*Balanites aegyptiaca* “Desert Date” of family *Zygophyllaceae* is a common wild plant found in many kind of habitats ,tolerating a wild variety of soil types from sand to heavy clay and climatic moisture levels. It is believed indigenous to all dry lands.

The root, stem bark ,fruit pulp and kernel cake of *Balanites aegyptiaca* have medicinal properties. Fruit is used in whooping cough also in leucoderma and other skin diseases. It is used as an oral hypoglycemic and an antidiabetic .An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in treatment of jaundice. It is also used to treat dysentery and constipation. Fruit is used to treat liver diseases and as a purgative and sucked by school children as a confectionary in some countries.

*Balanites aegyptiaca* contains steroids ( saponins, sapogenins, diosgenins ) used as raw material for industrial production of contraceptive pills, corticoids, anabolisants and other sexual hormones. The saponins occurring in roots ,woodchips and fruits facilitate their use for washing clothes..

Medicinal plants are rich source of secondary metabolites, but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary metabolites present in small quantities in specialized cells , but they possess significant biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical.

**Antioxidant** is simply a molecule that prevents another molecule from oxidizing. Since there are many processes in the body which result in oxidation , the intake of antioxidant is essential to counteract some of the negative results of the buildup of too many oxidized molecules in the body.

**Flavonoids** are a type of antioxidants , water soluble phenolic glycosides, which occur almost universally in higher plants. They are generally not synthesized by the animals. Flavonoids are easily recognizable as the pigments in flowers and fruits. They have multiple biological effects including antioxidant free radical scavenging abilities, anti-inflammatory , anticarcinogenic antiallergic, antiulcer, antihypototoxic, antiviral, antiinginal, antispasmylic etc. Their contribution to physiological functions such as seed maturation and dormancy has already been established ( Brenda, 1998 ).Their vital role is defences against pathogens and predators and physiological functions ( Winkel Shirley, 2001,2002 ).

Presence of flavonoids *in vitro* has been reported from many plant species like *Embilica officinalis* (*Kamal et al.*,1982 ),*Stevia nepifolia* (*Rajbhandari ,1984*), *Arachis hypogea* (...
tuberosa (Yadav, 2010), (Deepa, 2009), activated at 100°C.

Thin glass plates coated with silica gel G were dried, 2.2 Qualitative Analysis

layer (upper layer) was washed with distilled water to

with ethyl acetate in separating funnel. The ethyl acetate

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The developed plates were air dried and visualized under UV light (254 nm) which showed two and one fluorescent spots (in A. excelsa and B. egyptiaca respectively) in ethyl ether fraction (second) coinciding with those of the standard samples of quercetin (yellow, Rf 0.82), luteolin (yellow, Rf 0.78) and one spot in ethyl acetate fraction (third) in both A. excelsa and B. egyptiaca coinciding with those of the standard samples of kaempferol (deep yellow, Rf 0.93). The plates were also placed in a chamber saturated with ammonia vapors to observe the colors of the spots (quercetin–yellow, luteolin-yellow and kaempferol–deep yellow). On spraying the developed plates with 5% ethanolic ferric chloride solution two spots (quercetin,luteolin) and one fluorescent spot (quercetin) in A. excelsa and B. egyptiaca respectively were seen in ethyl ether fraction (second) and one spot in ethyl acetate fraction (kaempferol) in both A. excelsa and B. egyptiaca. The RF values were calculated as an average of the five replicates.

2.3 Preparative Thin Layer Chromatography (PTLC)

Glass plates (20 x 20 cm), thickly coated (wet thickness, 0.4-

0.5 mm) with silica gel ‘G’ (45 gm / 80 ml water), activated

at 100°C for 30 minutes and cooled at room temperature,

were used for preparative thin layer chromatography (PTLC).

The extract of both the fractions (second and third) of A. excelsa and B. egyptiaca were applied on separate plates and developed plates were air dried and visualized under UV light (254 nm). Each of the fluorescent spot coinciding with those of the standard reference compounds of quercetin, luteolin and kaempferol were marked. The marked spots were scrapped and collected separately along with the silica gel and eluted with ethanol. Each elute was then crystallized with chloroform.

The compounds thus isolated were subjected to colorimetry (for quantitative estimation), melting point (melting point apparatus, Toshiwall, India), UV maxima on a spectrophotometer (Carl-zeiss, Jena, DDR, VSU-2P) and Infra-red spectral (Perkin-Elmer, 337, Grating Infra-red spectrophotometer, using nujol or potassium bromide pellets) studies.

2.4 Quantitative Analysis

Spectrophotometry

Quantitative estimation of the identified flavonoids was carried out colorimetrically following the method of Kariyon et al., (1953) and Naghski et al., (1975) in case of quercetin as well as luteolin and of Mabry et al., (1970) in case of kaempferol. Stock solutions of quercetin, luteolin and
kaempferol were separately prepared by dissolving the authentic samples in methanol. Six concentrations (25 mg/ml to 150 mg/ml) of each of the standard samples were spotted on silica gel ‘G’ coated and activated plates. Separate plates for each of the concentrations of quercetin, luteolin and kaempferol were used and these chromatograms were developed in the same solvent system as used for qualitative method (n-butanol:acetic acid:water, 4:1:5; upper layer). Such developed chromatograms were air dried and visualized under UV light (254 nm). The fluorescent spots were marked and collected along with the absorbent in separate test tubes in methanol. The mixture was shaken vigorously, centrifuged and supernatents collected separately. The volume of elutes was made up to 10 ml by adding spectroscopic methanol. To each of these samples 3 ml of 0.1 M aluminium chloride was added stoppered tightly and the mixture shaken vigorously. Such tubes were kept at room temperature for 20 minutes. Five such replicates were prepared in each case and optical densities (O.D.) measured, using spectroscopic 20 colorimeter (Bausch and Lomb), set at 440 nm for quercetin, luteolin and at 423 nm for kaempferol against a blank (10 ml spectroscopic methanol + 3 ml of 0.1 M AlCl3). Regression curves for quercetin, luteolin and kaempferol were separately plotted in between their respective concentrations and optical densities, which followed Beer’s law.

Each of the ethyl ether and ethyl acetate extracts was dissolved in 1 ml of spectroscopic methanol and applied (0.1 ml) on silica gel ‘G’ coated plates along with authentic quercetin, luteolin and kaempferol markers and developed as above. Fluorescent spots coinciding with those of the reference compounds were marked, scrapped, eluted with methanol separately and the samples were prepared as detailed above. The optical density in each case was colorimetrically recorded as above. The amount of quercetin, luteolin and kaempferol in the samples were then determined (mg/100g.d.w.) by comparing with those of their respective standard regression curves. Five such replicates were examined and mean values were calculated (SE < 0.5 %).

3. Results and Discussion

Presence of Quercetin (Rf 0.82, m.p.309-3110 C,UV max 258, 373, yellowish blue with FeCl3) Luteolin (Rf 0.78, m.p.328°C, UV max 255,350, green with FeCl3) and Kaempferol (Rf 0.93, m.p. 271-273° C, UV max 268, 368 deep yellow to brown with FeCl3) have been identified, confirmed and measured quantitatively in all samples of unorganized cultures of A. excelsa while Quercetin and Kaempferol in B. egyptiaca. The characteristic IR spectral peaks were coinciding with those of their respective standard reference compounds of quercetin, luteolin and kaempferol in all samples.

Maximum GI was observed at the age of eight weeks in standardized (Sd) MS medium and standaized MS media supplemented with various concentrations (1,2,3 mg/L) of growth hormones (IAA and NAA) and salts (NaCl and KCl (10,20,30 mg/L) in A. excelsa and B. egyptiaca. Calli were harvested at maximum GI from all the samples separately in both plants.

It was observed that amount of flavonoids was increased in callus fed with growth regulators IAA and NAA. Increase was continuous from Sd MS medium to Sd MS medium fed with 1mg/L and then started decreasing continuously upto 3 mg/L IAA and NAA separately in both plant species. The amount calculated in calli fed with 2 and 3mg/L IAA and NAA was lower than amount of flavonoids present in calli grown on Sd MS medium. Maximum amount of flavonoids was calculated in calli fed with 1mg/L IAA (0.47+0.40+0.51=1.38 mg/100 g.d.w. and 0.45+0.48=0.93 mg/100 g.d.w. respectively ) and NAA(0.48+0.40+0.52=1.40 mg/100 g.d.w. and 0.44+0.48=0.92 mg/100 g.d.w. respectively) in A. excelsa as well as B. egyptiaca (Table 1).

In calli fed with salts KCl and NaCl, the amount of flavonoids was increased from Sd MS medium to calli fed with 10 mg/L and then decreased from 10 mg/L to 20 mg/L upto 30 mg/L in both plant species. Maximum amount was calculated in calli fed with 10mg/L NaCl (0.49+0.41+0.52=1.42 mg/L and 0.46+0.49=0.95 mg/L respectively) and KCl (0.48+0.40+52=1.40 mg/L and 0.46+0.48=0.94 mg/L respectively) in A. excelsa as well as B. egyptiaca (Table 2).

Amount of quercetin is higher than kaemferol in all the samples of both plant species and growth hormones have been proved to be better for increasing the flavonoid content. Luteolin is present only in A. excelsa and its amount is lowest.

4. Conclusion

Up to acertain concentration of growth regulators, the amount of flavonoids can be increased in vitro and it can be useful on large scale production.

References


Ajmer, Rajasthan.


Table 1: Effect of Growth Regulators On Flavonoid Content (mg/100 g.d.w.) IN A. excelsa AND B. aegyptiaca IN Vitro (At Maximum GI)

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Flavonoids</th>
<th>Sd MS Medium</th>
<th>Salts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1mg 2mg 3mg 1mg 2mg 3mg</td>
<td></td>
</tr>
<tr>
<td>A. excelsa</td>
<td>Kaempferol</td>
<td>0.46±0.05</td>
<td>0.47±0.06 0.45±0.06 0.40±0.04 0.48±0.04 0.44±0.05 0.41±0.06</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>0.38±0.04</td>
<td>0.40±0.05 0.35±0.03 0.31±0.06 0.40±0.04 0.37±0.03 0.30±0.03</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.50±0.06</td>
<td>0.51±0.06 0.48±0.07 0.45±0.05 0.52±0.04 0.49±0.03 0.43±0.05</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1.34±0.05</td>
<td>1.38±0.05 1.28±0.06 1.16±0.07 1.40±0.06 1.30±0.04 1.14±0.05</td>
</tr>
<tr>
<td>B. aegyptiaca</td>
<td>Kaempferol</td>
<td>0.42±0.06</td>
<td>0.45±0.07 0.42±0.05 0.36±0.05 0.44±0.05 0.40±0.06 0.34±0.04</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.46±0.04</td>
<td>0.48±0.04 0.45±0.05 0.39±0.06 0.48±0.06 0.44±0.04 0.37±0.05</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.88±0.06</td>
<td>0.93±0.04 0.87±0.04 0.75±0.05 0.92±0.05 0.84±0.04 0.71±0.05</td>
</tr>
</tbody>
</table>

Values are mean of five replicates ± SD
### Table 2: Effect of Salts on Flavonoid Content (mg/100 g.d.w.) IN *A. excelsa* AND *B. aegyptiaca* IN VITRO (At Maximum GI)

<table>
<thead>
<tr>
<th>Name of Plant</th>
<th>Flavonoids</th>
<th>Sd MS Medium</th>
<th>NaCl/L 10mg</th>
<th>KCl/L 10mg</th>
<th>NaCl/L 20mg</th>
<th>KCl/L 20mg</th>
<th>NaCl/L 30mg</th>
<th>KCl/L 30mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. excelsa</em></td>
<td>Kaempferol</td>
<td>0.46±0.04</td>
<td>0.49±0.04</td>
<td>0.44±0.06</td>
<td>0.40±0.04</td>
<td>0.48±0.05</td>
<td>0.44±0.08</td>
<td>0.38±0.06</td>
</tr>
<tr>
<td></td>
<td>Luteolin</td>
<td>0.38±0.06</td>
<td>0.41±0.07</td>
<td>0.37±0.06</td>
<td>0.33±0.04</td>
<td>0.40±0.06</td>
<td>0.36±0.06</td>
<td>0.30±0.04</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.50±0.08</td>
<td>0.52±0.05</td>
<td>0.49±0.07</td>
<td>0.46±0.05</td>
<td>0.52±0.03</td>
<td>0.48±0.06</td>
<td>0.45±0.08</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1.34±0.05</td>
<td>1.42±0.05</td>
<td>1.30±0.06</td>
<td>1.19±0.04</td>
<td>1.40±0.05</td>
<td>1.28±0.06</td>
<td>1.13±0.04</td>
</tr>
<tr>
<td><em>B. aegyptiaca</em></td>
<td>Kaempferol</td>
<td>0.42±0.04</td>
<td>0.46±0.06</td>
<td>0.43±0.04</td>
<td>0.38±0.05</td>
<td>0.46±0.04</td>
<td>0.42±0.04</td>
<td>0.35±0.05</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>0.46±0.07</td>
<td>0.49±0.06</td>
<td>0.46±0.03</td>
<td>0.40±0.02</td>
<td>0.48±0.03</td>
<td>0.45±0.05</td>
<td>0.38±0.05</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.88±0.06</td>
<td>0.95±0.05</td>
<td>0.89±0.02</td>
<td>0.78±0.03</td>
<td>0.94±0.02</td>
<td>0.87±0.04</td>
<td>0.73±0.05</td>
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</tbody>
</table>

Values are mean of five replicates ± SD