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ACUTE TOXICITY STUDY AND ANALGESIC ACTIVITY OF SUCCESSIVE EXTRACTS OF ROOTS OF *Flemingia macrophylla* (WILLD.)

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ABSTRACT

Flemingia (Willd.), a genus of the *Fabaceae* family, includes a wide range of herbs which are mostly dispersed in temperate and hilly areas. About 7 species are found in India, of which *Flemingia macrophylla (Willd.)* is the most commonly used as drug traditionally. It is an erect shrub with young branches found in hilly regions of Uttaranchal, in Dehradun and Western Ghats at the height 300-1700m from sea level. It is an important drug used in the treatment of pain of muscle, pain of body. Also employed in treatment of Kala azar, fever etc. *Flemingia macrophylla* has no reported literature of detailed pharmacological work so far. Hence, it has been decided to undertake pharmacological studies where acute toxicity study and analgesic study were performed with successive extracts Chloroform, Ethanol and Water. There was no sign of toxicity observed at the dose level of up to 6gm/kg body weight. Analgesic activity was shown by the Alcoholic and Chloroform extracts by the method of tail immersion as compared to standard drug diclofenac sodium 3mg/kg.

Key words: *Flemingia* (Willd.)**, Fabaceae,** *Flemingia macrophylla* **, Acute Toxicity Study.**

INTRODUCTION

Flemingia macrophylla (Willd.) Merrill (Fabaceae) is commonly known as "Bara Salpan, Bhalia and Kusant". It is an erect shrub with young branches with leaves 3-foliate; petioles long with angular but not winged, margins pubescent [1]. The traditional medical practices are an important part of the primary healthcare system in the developing world [2]. In the traditional system of medicine, the root of plant is used in pain of muscle, pain of body, also employed in the treatment of Kala azar, spleen peranagum, cholera, dysentery, fistula ani, prolepsis ani, smallpox, fever and gastrosis [3,4]. Whole plant is used in the treatment of cold, skin diseases, stomachache. Seeds are used as saffron substitute. *F. macrophylla* has reported literature about the leaves and aerial parts, leaves found to contain 5, 7, 4'-trihydroxy-6-3'-diprenylisoflavone, 5, 7, 4'-trihydroxy-6-8-diprenisoflavone and flemingin-D also it was found to contain three new flavanoids, fleminginin, flemingichromone, flemingichalcone and chalcone which were isolated from the active fractions of aerial parts. *F. macrophylla* also has reported literature about the stems, mathanolic extract of stems of *F. macrophylla* found to contain fleminone a flavanone [5,6]. According to the World Health Organization (WHO) as many as 80% of world's population depends today on traditional medicine for their primary health care needs [11].

MATERIALS AND METHODS

Plant Material

Fresh samples of roots of *F. macrophylla* (Willd.) Merrill (Fabaceae) were collected from Ranikhet in the month September, 2009 and was authenticated through Regional Research Institute Tarikhet (Ranikhet) Uttarakhand having the specimen no. RKT14360 and the herbarium was preserved. Roots were washed thoroughly to remove impurities and few samples were dried in hot air oven at 55˚C. Then grounded to yield fine powder which was further subjected for the extraction processes.

Preparation of plant extract

The extraction process was carried out by Hot Continuous Extraction (Soxhlet) where the solvents were Chloroform and Alcohol used successively in the order of increasing polarity and finally the drug was extracted with water by the process of decoction [7].

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i. The yield of chloroform extract after the 33 hrs. continuous extraction was found to be **1.11%**.
ii. The yield of alcoholic extract after the 67 hrs. continuous extraction was found to be **11.04%**.
The yield of aqueous extract after the extraction was found to be **2.5%**.

Experimental animals

Acute toxicity study

Male Swiss albino mice of body weight from 25-30 g were procured from IFTM Institute, Moradabad. The animals were housed in polypropylene cages in air conditioned room with controlled temperature and alternating 12 hour periods of light and dark were maintained. The animals were acclimatized to standard laboratory conditions prior to experimentation. The guidelines issued by Institutional Animal Ethics Committee of IFTM College of Pharmacy, Moradabad.

Analgesic study

Male Wistar rats, weighing 250-300 gm were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of College of Pharmacy, I.F.T.M, Moradabad, U.P, India.

Acute Toxicity study

Acute Toxicity study of *Flemingia macrophylla* was carried out by using mice as the experimental model. The study was carried out to assess the acute toxicity of the plant extract made by the extraction of plant material with continuous hot extraction (Soxhletion). The study was carried out as per the details laid down in OECD guidelines 420 viz, fixed dose procedure (Evident toxicity). More clinical observation such as condition of fur, damage area of skin, subcutaneous swelling or lumps, abdominal detention, eye dullness, eye opacity, pupil diameter, ptosis (drooping of upper eyelid), colour and condition of faeces, wetness or soiling of perineum, condition of teeth and breathing abnormalities should be recorded as indication of toxicity [8].

Analgesic activity

48 rats were used in the study with 8 groups of six rats weighing 250-300gm were administered orally, diclofenac (3 mg/kg), extract (300 and 600 mg/kg) for different successive extracts and control group of six rats. The distal part of the tails of the animals was immersed in hot water maintained at 55.0± 1.0 °C. The time taken to withdraw the tail was noted as reaction time [9]. A cut off time of 10 sec was maintained at 55 °C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45 and 60 min after treatment, respectively.

Composition of diet

The animals were fed on the standard pellet diet, and water was given *ad libitum*. The standard pellet diet comprised 20% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates).

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis of the data were carried out using One way ANOVA followed by Dunnett's test and results were considered significant when $p < 0.05$ for analgesic activity.

RESULT AND DISCUSSION

Acute Toxicity Study Protocol:-

1. Animal species/ strain	Albino Swiss mice.
2. Sex	Male.
3. Body weight	24–30.
4. Animal procured from	IFTM, College of Pharmacy, Moradabad.
5. No. of doses groups	12
6. Animals per group	5
7. Route of administration	Oral via gauge.
8. Vehicle of administration	Distilled water
9. Volume of administration	Not more than 1ml as combined volume of plant sample and vehicle.
10. Dosing details	Refer to dosing table (Table 1).
11. Observation period	14 days post dose and 7 days prior to dosing.

Clinical Observation

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study as compared to control. Any changes or abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of whole plant powder as slurry. The clinical observation detailed below is in general for the plant material under investigation [10].

1. Condition of fur	Normal
2. Damage area of skin	Normal
3. Subcutaneous swelling or lumps	Normal
4. Abdominal detention	Normal
5. Eye dullness	Normal
6. Eye opacity	Normal
7. Pupil diameter	Normal
8. Ptosis (drooping of upper eyelid).	Normal
9. Colour and condition of faeces	Normal
10. Wetness or soiling of perineum	Nil
11. Condition of teeth	Normal
12. Breathing abnormalities	Normal

Body weight changes

Body weight is an important factor to monitor the health of the animal. The loss of body is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10% or more reduction in body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weight for all the 14 days as compared with the 0 day it thus indicating no signs of toxicity.

Food and water consumption

There was no significant change in food and water consumption.

Mortality

Mortality is the main criterion in assessing the acute toxicity (LD50) of a drug. There was no mortality recorded even at the highest dose level i.e.6g/kg body weight of all the groups.

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the male Wistar rats from all the dose groups. There was no mortality recorded even at the highest dose level i.e.6g/kg body weight, which proves that all the extracts of *Flemingia macrophylla* viz. alcohol, aqueous, chloroform have no toxic effect in male Wistar rats. The results have indicated that this plant is safe.

Analgesic study

The analgesic activity of the extracts was done with the dose regimen of 300 mg/kg and 600 mg/kg. Analgesic activity for all the extracts was done but the significant activity was shown only for the dose regimen of 600 mg/kg whereas 300 mg/kg dose only shown the significance in the 120^{th} minute. In the case of the dose regimen of 600 mg/kg chloroform extract was found to be more effective than ethanolic and aqueous extracts. Ethanolic extract at 600 mg/kg showed significant activity at 15^{th}, 90^{th} and 120^{th} minute of the experiment; aqueous extract showed the significant activity in 30^{th}, 90^{th} and 120^{th} minute of the experiment and chloroform extract showed the activity at the 15^{th}, 30^{th}, 90^{th} and 120^{th} minute of the experiment. The significance study was done by ANOVA followed by Dunnett's test as compared to control.

The ethanolic extract was found to contain flavonoids and steroids; aqueous extract was found to contain flavonoids, carbohydrates, tannins and phenolic compounds and saponins; chloroform extract was found to contain carbohydrates, steroids and tannins and phenolic compounds. The analgesic activity was showed by the presence of one or more group of compounds. The results are shown below.

Table 1. Dose regimen of acute toxicity study of roots of *Flemingia macrophylla* (Willd.)

Group	Sex	Extract	Dose. g/kg. body weight	No. of animal used	Total volume administered
I	Male	Alcohol	0.5	5	0.5
II	Male	Alcohol	1	5	0.5
III	Male	Alcohol	2	5	0.5
IV	Male	Alcohol	4	5	0.5
V	Male	Alcohol	6	5	0.5
VI	Male	Aqueous	0.5	5	0.5
VII	Male	Aqueous	1	5	0.5
VIII	Male	Aqueous	2	5	0.5
IX	Male	Aqueous	4	5	0.5
X	Male	Aqueous	6	5	0.5
XI	Male	Chloroform	0.5	5	0.5
XII	Male	Chloroform	1	5	0.5
XIII	Male	Chloroform	2	5	0.5
XIV	Male	Chloroform	4	5	0.5
XV	Male	Chloroform	6	5	0.5

Note: Aqueous and alcoholic extract dissolved in water, chloroform extract dissolved in Dimethylsulphoxide (DMSO).

Table 2. Acute toxicity study of root of *Flemingia macrophylla* (Willd.) Merrill–Body weight (gm)

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	29.8	24	30	30	30	30	24	30	24.2	30	30	30	24.8	29.9	26
1	29.4	24.2	29.9	29.9	29.9	30	24.2	29.9	24.8	29.5	29.9	30	24.2	29.9	25.8
2	29.8	24.8	29.9	30	29.9	29.9	24.8	29.9	24.2	29.8	29.9	29.9	25	29.8	25.9
3	29.8	24.2	29.9	30	30	29.9	24.2	29.9	25	29.4	30	29.9	24.6	30	25.4
4	29.8	25	29.8	29.8	29.8	29.8	25	29.8	24.6	29.8	29.8	29.8	24.7	28.9	25.7
5	30	24.6	29.6	29.8	30	30	24.6	29.6	24.7	29.8	30	30	25	30	25
6	30	24.7	29.5	30	28.9	28.9	24.7	29.5	30	29.8	28.9	28.9	25.4	29.6	25
7	30	25	30	30	30	30	25	30	30	30	30	30	25.7	29.5	25.4
8	29.9	25.4	30	30	29.9	29.9	25.4	30	30	30	29.9	29.9	25	30	25.7
9	30	25.7	30	29.2	30	30	25.7	30	29.2	30	30	30	25.4	30	25
10	29.9	25	29.9	29.5	29.9	29.9	25	29.9	29.5	29.5	29.9	29.9	25.7	29.9	25.4
11	30	25.9	30	29.7	30	30	25.9	29.8	29.7	30	30	30	25	29.8	25.6
12	28.9	26	28.9	29.8	29.8	28.9	26	29.6	29.8	30	29.8	28.9	25.9	29.6	25.7
13	30	25.8	30	29.9	29.9	30	25.8	29.5	29.9	29	29.9	30	26	29.5	26.2
14	30	25.9	30	30	30	30	25.9	29.9	30	30	30	30	26	30	26.3

Note: Group (I, II, III, IV, V)- Alcohol(0.5, 1, 2, 4, 6 gm/kg), Group(VI, VII, VIII, IX, X)- Aqueous (0.5, 1, 2, 4, 6 gm/kg), Group(XI, XII, XIII, XIV, XV)- Chloroform(3, 5 gm/kg), average weight of animals and number of animals in each group is five.

Table 3. Acute toxicity study of root of *Flemingia macrophylla* (Willd.) Merrill–Food intake (gm)

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	20.3	20	16	20	20	15	22	17	20	19	17	17	19.3	22	17
1	17	15	17	20	21	15	18	16.5	18	15	20	16.2	20	18	19
2	19	17	17	19	22	17	20.5	17	15	19	17	17	19.2	22	17.2
3	15.5	20.5	16	20	18	19	23	16.5	19	15	20.1	16.2	20.1	18	19
4	18	15	16.3	18	20	15.3	20.5	16.3	20	18	15.5	16	18.3	20.2	15.5
5	22	15	18	15.5	23	16	21	18	20	22	15.3	18.5	15	23.5	16
6	15.5	18	19	19.5	20	19.5	19	19	18.5	15.5	18	19.5	19.5	20	19.5
7	22	20	19	20.2	21	20.2	21.2	19.1	20.1	22	20	19	20	21	20
8	20.3	20.5	18.5	20	19	20	19	18	20	20	20	18	20	19.5	20.3
9	21.2	18.2	16	18	21	17	19	16	18	21	18	16.5	18	21.5	17
10	17	16	20.3	20.2	19	19	22.2	20	19.2	17	16	20.5	20	19.5	19.5
11	21	20	20	20	22.3	20	19.5	20	20	17	16.3	20	20.2	19	19
12	18	20	18	18.5	19	20.5	21	20	18	21	20	20	20.1	22	20.5
13	20	19.3	19	19.3	21.2	19	20.3	18.5	16.1	18.1	20.2	18	18	19.2	20
14	21.5	17	17.3	20	19	18	20.3	19.5	20.5	20.3	19.2	19.1	19.2	21.5	19.2

Note: Group(I, II, III, IV, V)- Alcohol(0.5, 1, 2, 4, 6 gm/kg), Group(VI, VII, VIII, IX, X)- Aqueous(0.5, 1, 2, 4, 6 gm/kg), Group(XI, XII, XIII, XIV, XV)- Chloroform(3, 5 gm/kg), and the values are expressed in weight of food consumed by each group from a known weight of food provided, number of animal in each group is five.

Table 4. Acute toxicity study of successive extracts of roots of *Flemingia macrophylla* (Willd.) Merrill. – Water intake (ml)

Days	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
0	190	200	190	220	190	180	220	170	190	215	190	185	190	200	200
1	185	110	180	200	200	190	200	175	200	210	200	190	200	190	220
2	200	190	180	190	200	160	190	195	200	190	200	170	200	210	210
3	195	200	150	210	220	200	210	205	220	210	215	195	220	190	190
4	185	200	190	190	210	220	190	190	210	185	195	220	210	170	210
5	215	150	180	170	190	180	170	195	190	170	190	180	190	190	210
6	145	170	200	190	210	190	190	200	210	195	205	190	210	200	205
7	210	130	190	200	210	200	200	195	210	205	210	200	210	200	210
8	205	205	170	185	205	195	200	195	200	200	205	190	205	190	170
9	185	190	150	190	210	190	190	190	210	180	210	190	210	200	190
10	180	160	200	200	180	190	200	200	180	200	180	190	180	220	200
11	200	200	190	220	200	210	220	200	200	210	200	210	200	180	185
12	190	180	170	180	180	180	180	185	180	180	180	180	180	190	160
13	180	190	190	190	180	190	190	185	180	190	180	190	180	210	200
14	205	170	190	210	170	190	210	205	170	205	170	190	170	185	180

Note: Group (I, II, III,IV,V)- Alcohol (0.5, 1, 2, 4, 6 gm/kg), Group (VI, VII, VIII, IX, X)-Aqueous (0.5, 1, 2, 4, 6 gm/kg), Group (XI, XII, XIII, XIV, XV)- Chloroform (0.5, 1, 2, 4, 6 gm/kg), average volume of water intake by each group and number of animals in each group is five.

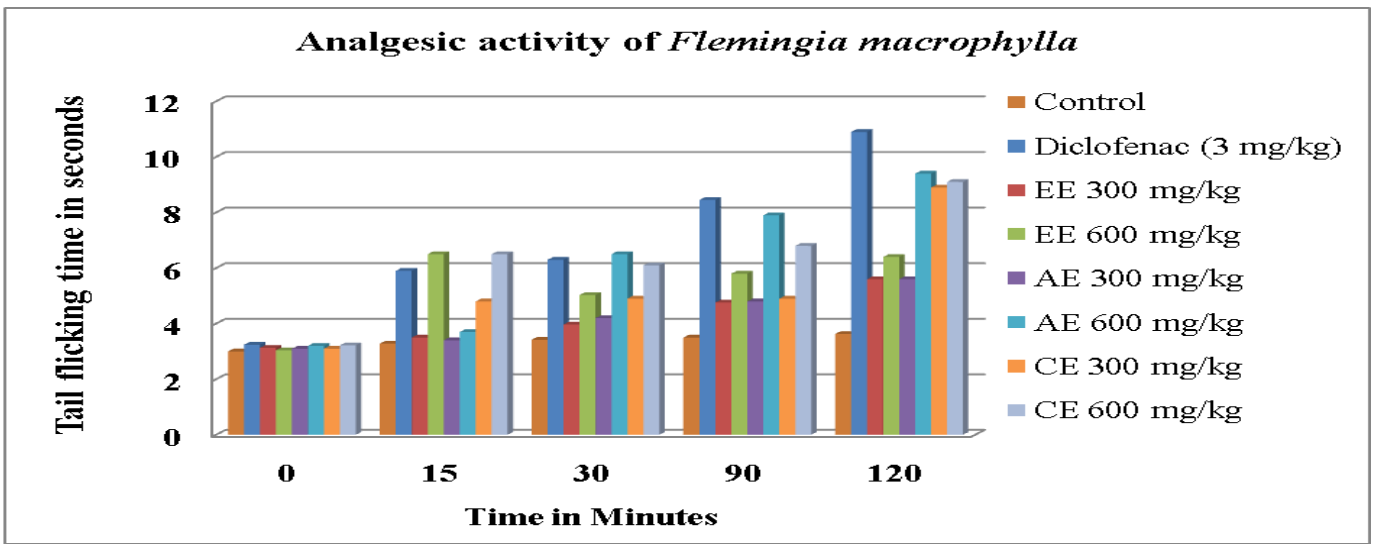
Table 5. Analgesic effects of different successive extracts of *Flemingia macrophylla* Willd. with Tail immersion method

Time interval (Minutes)	Reaction time in seconds (Mean ± SEM)							
	Control	Diclofenac (3 mg/kg)	EE 300 mg/kg	EE 600 mg/kg	AE 300 mg/kg	AE 600 mg/kg	CE 300 mg/kg	CE 600 mg/kg
0	3±0.15	3.24±0.04	3.13±0.07	3.04±0.19	3.10±0.12	3.2±0.09	3.1±0.02	3.22±0.05
15	3.28±0.114	5.9±0.4***	3.5±0.5*	6.5±0.8***	3.4±0.43*	3.7±0.4*	4.8±0.29*	6.5±0.6***
30	3.42±0.09	6.3±0.14***	3.97±0.4*	5.03±0.82*	4.2±0.6*	6.5±0.07***	4.9±0.2*	6.1±0.4***
90	3.5±0.09	8.45±0.4***	4.76±0.3*	5.8±0.4***	4.8±0.3*	7.9±0.4***	4.9±0.6*	6.8±0.9***
120	3.63±0.11	10.9±0.7***	5.6±0.2***	6.4±0.3***	5.6±0.4***	9.4±0.2***	8.9±0.2***	9.1±0.2***

in rats

Values are expressed in Mean ± S.E.M.; n=6; significance at P>0.05*, P<0.05**, P<0.01*** *as compared to the control* (ANOVA followed by Dunnett's Test). **EE**- Ethanolic extract; **AE**- Aqueous extract; **CE**- Chloroform extract.

Fig 1. Effects of different successive extracts of *Flemingia macrophylla* Willd. roots at different doses (300 and 600 mg/kg body weight) as compared to control



Diclofenac: Diclofenac sodium, **EE:** Ethanolic extract, **AE:** Aqueous extract, **CE:** Chloroform extract.

CONCLUSION

From the results of this study it is observed that there is no significant change in body weight, food and water consumption by the Swiss mice from all the dose groups. There was no mortality recorded even at the highest dose level i.e. 6gm/kg body weight, which proves that all the extracts of *Flemingia macrophylla* viz. alcohol, aqueous, chloroform have no toxic effect in Albino Swiss mice. The analgesic activity of the dose regimen of 600 mg/kg chloroform extract was found to be more effective than ethanolic and aqueous extracts. Ethanolic extract at 600 mg/kg showed significant activity at 15^{th}, 90^{th} and 120^{th} minute of the experiment; aqueous extract showed the significant activity in 30^{th}, 90^{th} and 120^{th} minute of the experiment and chloroform extract showed the activity at the 15^{th}, 30^{th}, 90^{th} and 120^{th} minute of the experiment. The results have indicated that this plant is safe and can be used for efficacy studies for different activities at the effective dose of 200, 300, 400 and 500 mg/kg. The ethanolic extract was found to contain flavonoids and steroids; aqueous extract was found to contain flavonoids, carbohydrates, tannins and phenolic compounds and saponins; chloroform extract was found to contain carbohydrates, steroids and tannins and phenolic compounds. The analgesic activity was showed by the presence of one or more group of compounds.

REFERENCE

1. Kirtikar KR & Basu. *Indian Medicinal Plants*, International Book Distributors, vol 2, 2005, 85.
2. Sheldon JW, Balick MJ, Laird SA. Medicinal plants: can utilization and conservation coexist? Advances in Economic Botany. *Economic Botany*; 12, 1997, 1-104.
3. Bakshi DNG, Sensarma P, Pal DC. *A Lexicon of Medicinal Plants in India*, Vol 2, Naya Prakashan Calcutta, 2001, 126.
4. Johnson. Ethnobotany Desk Reference, CRC Press, Washington DC, 2003, 352.
5. Nageshwara K, Srimannarayana G. Fleminone a flavanone from the stems of *Flemingia macrophylla*. *Journal of Phytochemistry*, 22(10), 1983, 2287-2290
6. Nageshwara K, Srimannarayana G. Flemiphyllin an isoflavone from stems of *Flemingia macrophylla*. *Journal of Phytochemistry*, 23(4), 1984, 927-929.
7. Mukherjee PK. *Quality Control of Herbal Drugs*, Business Horizons Publishers; 2002, pg 398.
8. Angel Sánchez-Lamar, Gladys Fonseca, Jorge Luis Fuentes, Renata Cozzi, Enrico Cundari, Mario Fiore, Ruggero Ricordy, Paolo Perticone, Francesca Degrassi and Rosella De Salvia. *Studies on the toxicity of Punica granatum* L. (Punicaceae) whole fruit extracts. *Journal of Ethnopharmacology*, 2003; 89, 2003, 295-300.
9. Kar DM, Nanda BK, Pradhan D, Sahu SK and Dash GK. Analgesic and antipyretic activity of fruits of *Martynia annua* Linn. *Hamdard Medicus*, 47(1), 2004, 32-35.
10. Butler LG, Rogler JC. Biochemical mechanisms of the antinutritional effects of tannins. In: Ho CT, Lee, CY, Huang MT. (Eds.), Phenolic Compounds in Food and their Effects on Health I. *American Chemical Society, Washington, DC*, 1992, 298-304.
11. Hassan Azaizeh, Bashar Saad, Edwin Cooper and Omar Said. Ethnomedicinal knowledge of local Arab practitioners in the Middle East Region. *Fitoterapia*, 74, 2003, 98-108.