Investigation of Nutritional Values and Some Bioactivities of Ocimum Basilicum L. (Thai Pin Sein)

Kay Khine Nyunt [1], Kay Thi Khine[2] , Ni Ni Than[3]

Abstract

Determination of nutritional values of *Ocimum basilicum* L. have been carried out by AOAC method, resulting 12.56% of moisture,12.79% of ash, 3.72% of fat, 10.54% of fiber, 27.01% of protein,33.38% of carbohydrate, and 275.04kcal/100g of energy value. Total phenolic content of EtOH extract 401.01 µg GAE/mg and watery extract 573.63 µg GAE/mg were observed. The total flavonoid content of EtOH extract 26.85 µg QE/mg and H_2O extract 74.07 µg QE/mg were observed. Antioxidant activity of *O. basilicum* was done by DPPH assay method. IC_{50} value of watery and ethanol extracts are 8.78 and 9.68 µg/mL respectively. The antimicrobial activity of polar and non -polar extracts of *O. basilicum* are tested on six microorganisms by agar well diffusion method. Among the tested extracts, EtOH extract inhibited all species of tested microorganisms with the inhibition zone diameters in the range between11-14mm. However, EtOAc extract inhibited four species namely, *BacilusSubtilis, Staphylococus aureus, Candida albicans* and *Escherichia coli.* PE and H_2O extracts of *O. basilicum* do not show antimicrobial activity on six tested microorganisms.

Keywords: *Ocimum basilicum* L., nutritional values, antioxidant activity, antimicrobial activity

Introduction

Ocimum basilicum L. (Thai Pin Sein) belong to the family lamiaceae. The genus *Ocimum* L. comprising of more than 150 species are grown widely throughout temperature regions of the world. *O.basilicum* L. commonly is called as Sweet Basil (Poonkodl, 2016). Many of the plant used today were known to the people of ancient culture throughout the world and were highly considered for their preservative and medicinal powers. Basil is one of the species used for the commercial seasoning. It is commonly known that the presence of essential oils and their composition determine the specific aroma of plants and the flavour of the condiments. The present of leaves of *O.basilicum* L. have been selected for chemical and biological studies due to its medicinal importance. There are many types, some large and some small, with a range of leaf colour from green to purple up to variated. *O. basilicum* L. is widely in the world which includes annual and perennial herbal plants as well as shrubs from tropical and subtropical zones (Haque *et al*., 2005). *Ocimum basilicum* L. have antioxidant activity, antimirobical activity, antiviral activity, anticarcinogenic, larvicidal agents and hypoglycaemic effect. Its leaves are often fragrant, aromatic and are also used as an expectorant. Decoction of the leaves, given in gastric and hepatic disorders and is useful in catarrh, bronchitis, in cough (due to heat), acts as diuretic as well as tonic for stomach (Rubab *et al.,* 2017).

[1] Lecturer, Dr, Department of Chemistry, University of Yangon
[2] MSc candidate , Department of Chemistry, University of Yangon
[3] Professor and Head, Dr, Department of Chemistry, University of Yangon

Botanical Aspect of *Ocimum basilicum* L.

Family	:	Lamiaceae
Genus	:	*Ocimum*
Species	:	*basilicum*
Botanical name	:	*Ocimum basilicum* L**.**
Myanmar name	:	Thai Pin Sein
English name	:	Sweet Basil (Rubab *et al*., 2017)

Materials and Methods

Sample Preparation and Identification

Several samples of *O. basilicum* L. *were* collected from Thiri Mingalr Market, Yangon Region in December, 2018. The leaves were chipped into very small pieces and allowed to dry well. The dried pieces were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture changes and other contaminations. The dried powdered sample was used for chemical and biological investigations.

Determination of Nutritional Values in Plant Samples

The nutritional values such as moisture, ash, fat, fiber and protein *Ocimum basilicum* L. of leaves were examined by AOAC method at Ministry of Agriculture, Livestock and Irrigation,Small Scale Industries Department, North Oakkalapa, Yangon, Myanmar.

Determination of Total Phenol Content by FCR Method

One of the antioxidative factors, total phenolic content (TPC) was measured spectrophotometrically according to the Folin-Ciocalteau method (Mamta *et al.,* 2013).

Procedure

(i) Determination of total phenol content as gallic acid equivalent in sample

The total phenolic content (TPC) in each sample was estimated by Folin-Ciocalteau method according to the procedure described by Saxena (2013). First, 0.5 mL of prepared extract solution was mixed with 0.5 mL of methanol. Then, 5 mL of FCR reagent (1:10) was added to the mixture and incubated for 5 minutes. 4 mL of 1 M sodium carbonate solution was added to each tube and the tubes were kept at room temperature for 2 hours and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. Total phenolic content was estimated as milligram gallic acid equivalents per gram of different extract (mg GAE/ g). The TPC contents of all tested samples are shown in Table 2 and Figure 2.

Determination of Total Flavonoid Content by Aluminium Chloride Colorimetric Method

Total flavonoid content (TFC) was measured spetrophotometrically according to the $AlCl_3$ colorimetric method.

Procedure

The total flavonoid content (TFC) in each sample was estimated by Aluminium Chloride colorimetric method according to the procedure. Each extracts solution (100 µg/mL) was mixed with 1.5 mL of methanol, 0.1mL of 1% $AlCl_3$ solution and 2.8 mL of distilled water. The absorbance of reaction mixture was read at λ_{max} 415 nm. Total flavonoid content was estimated as milligram quercetin equivalent per gram (mg QE/g) of extract. The TFC contents of all tested samples are shown in Table 3. and Figure 3.

Determination of Antioxidant Activity of Crude Extracts of Leaves of *Ocimum basilicum* L. (Thai Pin Sein) by DPPH Free Radical Scavengin Assay

The free radical scavenging activity of crude extracts *O. basilicum* L. (Thai Pin Sein) was measured using DPPH free radical scavenging assay (Marinova and Batchvarov, 2011).

Procedure

DPPH radical scavenging activity of ethanol and water extracts of leaves of *O. basilicum* L. (Thai Pin Sein) was determined by UV-visible spectrophotometer (Marinova and Batchvarov, 2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002% DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of different concentrations (6.25, 12.5, 25, 50, 100 and 200 µg/mL) of tested sample was measured at 517 nm using UV-7504 spectrophotometer. Absorbance measurements were done for three times for each concentration and the mean value so obtained were used to calculate percentage of radical scavenging activity (% RSA) by the following equation. % RSA of crude extracts of leaves of *O. basilicum* L. and standard butylatedhydroxy toluene (BHT) results are shown in Table 4.

$$\% \text{ RSA} = [A_{DPPH} - (A_{Sample} - A_{Blank})/ A_{DPPH}] \times 100$$

Where;

$\% \text{ RSA}$ = % radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample + DPPH solution

A_{Blank} = absorbance of sample + EtOH solution

The antioxidant power (IC_{50}) is expressed as the test substances concentration (μg/mL) that result in a 50 % reduction of initial absorbance of DPPH solution and that allows to determine the concentration. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program. The standard deviation was also calculated by the following equation. IC_{50} values of crude extracts of leaves of *Ocimum basilicum* L. (Thai Pin Sein) and standard butylated hydroxyl toluene (BHT) are shown in Table 5 and Figure 4.

Investigation of Antimicrobial Activity of Different Crude Extracts of *Ocimum basilicum* L.

PE, EtOH, EtOAc and H_2O extracts of the leaves of *O.basilicum* L. were detected by Myanmar Pharmaceutical Industrial Enterprise, Research Department, Yangon.

The microbial strains used in this antimicrobial assay were as follows:

(a) *Bacillus subtilis*

(b) *Staphylococcus aureus*

(c) *Pseudomonas aeruginosa*

(d) *Bacillus pumilus*

(e) *Candida albicans*

(f) *Escherichia coli*

Procedure

PE extract of Thai Pin Sein (1 g) was dissolved in PE (1 mL), EtOAc extract (1 g) was dissolved in EtOAc (1 mL), EtOH extract (1 g) was dissolved in EtOH (1 mL), and H_2O extract (1 g) was dissolved in H_2O (1 mL). All microbicalogical media tested were prepared according to the procedure of the manufacturer by using aspect condition. Agar well diffusion method was employed for determining antimicrobial activity of the extracts. Two small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sample placed in each hole was 0.1 mL. The samples, namely PE, EtOAc, EtOH and H_2O solution of *O. basilicum* L. leaves were tested. The Petri dish was ten incubated at 37º C for 48 hours, and the diameter of clear inhibition zone appeared around the hole were measured (Priestaman and Edward, 1954). The results are described in Figure 5, 6 and Table 6.

Results and Discussion

Nutritional Values of Leaves of *Ocimum basilicum* L. (Thai Pin Sein)

People and other living organisms need certain substances of nutrient to survive. Nutritional values such as moisture, ash, protein, fibre, fat in the leaves were determined and the results are shown in Table 1. Leaves of Thai Pin Sein contains moisture (12.56 %), ash (12.79 %), fat (3.72 %), fiber (10.54 %), protein (27.01 %), carbohydrate (33.38 %) and energy values (275.04 kcal/100g). From these data, it can be clearly seen that the carbohydrate content is the highest, followed by the protein

content leading to high food energy value. The moisture, ash, fiber contents are not found to be different, but the fat content is the lowest in *O. basilicum* L.

Table 1. Nutritional Values of Leaves of *Ocimum basilicum* L.*

No	Nutrients	Contents %
1	Moisture	12.56
2	Ash	12.79
3	Fat	3.72
4	Fiber	10.54
5	Protein	27.01
6	Carbohydrate	33.38
7	Energy values	275.04 kcal/100g

*Ministry of Agriculture, Livestock and Irrigation Small Scale Industries Department

Total Phenol Content of Crude Extracts from the Leaves *Ocimum basilicum* L.

In this study, a significant linear correlation was observed between the values for the total phenol content and antioxidant activity. The high contents of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The total phenol contents of watery and ethanol extracts from the leaves of Thai Pin Sein are shown in Table 3.6. According to the results, the higher TPC (μg GAE/mg) was detected in watery extract (573.63 μg GAE/mg) than ethanol extract (401.01 μg GAE/mg). This means that phenolic compounds were more soluble in water. Watery extract of *O. basilicum* L. was a good source of phenolic compounds which are effective on antioxidant activity. Comparison of TPC in watery and ethanol extracts from the leaves of Thai Pin Sein are represented by bar graph in Figure 2.

Table 2. Total Phenol Content (TPC) in H_2O and EtOH Extracts from the Leaves of *Ocimum basilicum* L.

No	Tested samples	Total Phenol Contents (μg GAE/mg)
1	Water	573.63
2	Ethanol	401.01

GAE = Gallic acid equivalent

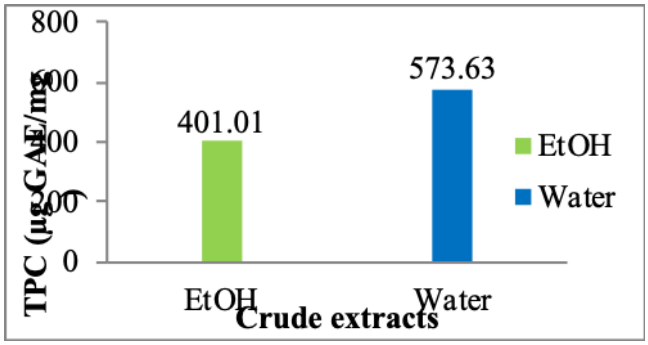


Figure 2. Total phenolic contents of watery and ethanol extracts of leaves of *Ocimum basilicum* L. (Thai Pin Sein)

Total Flavonoid Content of the Crude extracts of Leaves of *Ocimum basilicum* L.

In this study, high flavonoid contents have been found to exert high antioxidant potential. The total flavonoid content of water and ethanol extracts of leaves of *O. basilicum* L. are shown in Table 3 and Figure 3. According to these result, the total flavonoid content of watery extract (74.07 µg QE/mg) was higher than that of EtOH extract (26.85 µg QE/mg).

Table 3. Flavonoid Contents of Watery and Ethanol Extracts of *Ocimum basilicum* L. (Thai Pin Sein)

No	Test samples	Total Flavonoid Contents (µg QE/mg)
1	Watery	74.07
2	Ethanol	26.85

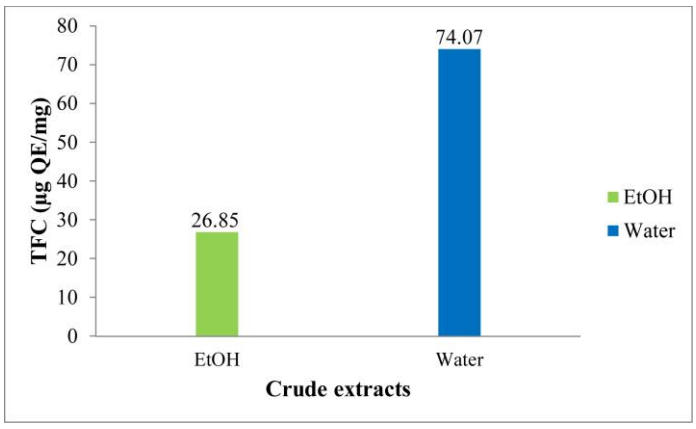


Figure 3. Total flavonoid contents of watery and ethanol extracts of leaves of *Ocimum basilicum* L. (Thai Pin Sein)

Antioxidant Activity of Ethanol and Water Crude Extracts of Leaves *Ocimum basilicum* L. (Thai Pin Sein) by DPPH Radical Scavenging Assay

From the experimental results, leaves of Thai Pin Sein were found to have antioxidant activity. IC_{50} values of ethanol and water extracts are 9.68 and 8.71 μg/mL respectively. Antioxidant potency of ethanol and water extracts were lower than that of standard butylated hydroxytoluene (IC_{50} = 6.85 μg/mL). The IC_{50} values of ethanol and water crude extracts of leaves and standard butylated hydroxytoluene are shown in Table 5 and Figure 4.

Table 4. Radical Scavenging Activity (% RSA) of H_2O and EtOH Extracts from Leaves of *Ocimum basilicum* L. and Standard BHT

Extracts	% RSA±SD of different concentrations (μg/mL)					
	6.25	12.5	25	50	100	200
Watery	44.35 ± 0.88	58.69 ± 0.63	70.07 ± 0.50	73.65 ± 0.25	81.89 ± 0.76	95.07 ± 0.63
75% ethanol	48.92 ± 0.76	50.89 ± 0.50	61.64 ± 0.25	72.93 ± 0.25	84.05 ± 0.25	96.32 ± 0.38
BHT(Std.)	49.11 ± 1.31	58.31 ± 0.87	68.69 ± 1.09	82.05 ± 1.31	91.10 ± 0.43	96.59 ± 1.97

Table 5. IC_{50} Values of Crude Extracts from Leaves of *Ocimum basilicum* L. and Standard BHT

Samples	IC_{50} (μg/mL)
H_2O	8.71
EtOH	9.68
BHT	6.85

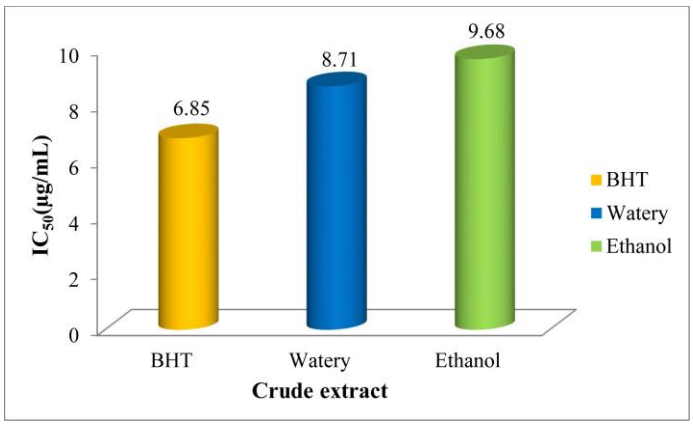
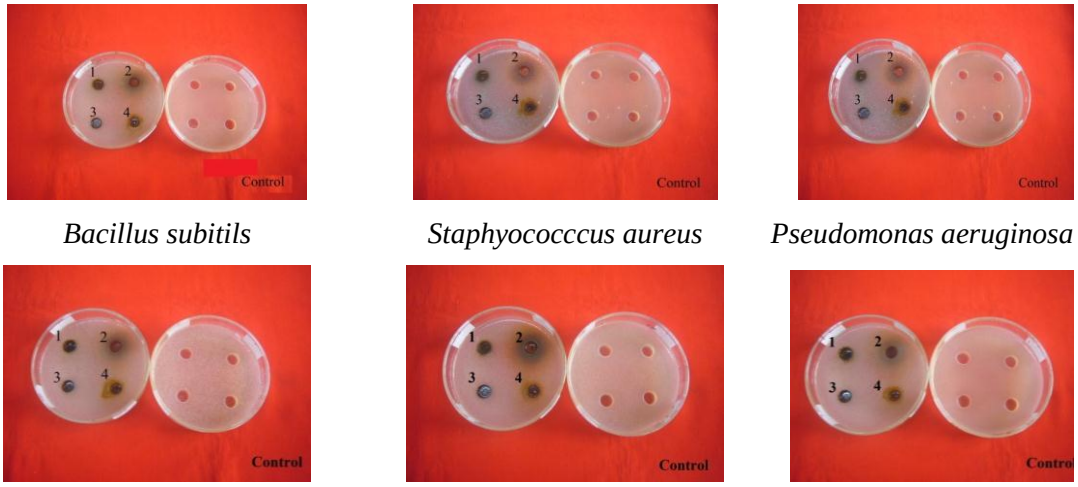


Figure 4. IC_{50} values of crude extracts from Thai Pin Sein and standard BHT

Screening of Antimicrobial Activity of the Leaves of *Ocimum basilicum* L.

According to the results, EtOH extract of the leaves of *O. basilicum* L. inhibited all species of tested microorganisms with the inhibition zone diameters in the range between 11−14 mm. However, EtOAc extracts inhibited four spices of microorganisms namely *Bacillus subtilis, Staphylococus aureus, Candida albicans and Escherichia coli.* However, H_2O extracts of *O. basilicum* L. do not show antimicrobial activity on all test microorganisms. The results are described in Figure 5,6 and Table 6.

Therefore, EtOH and EtOAc extracts of the leaves of *O.basilicum* L. showed low antimicrobial activity on tested microorganisms. However, it may be used for the treatment of diseases infected by the microorganisms such as diarrhoea, dysentery, urinary tract and wound infections.



Bacillus subitils *Staphyococccus aureus* *Pseudomonas aeruginosa*

Bacillus pumilus *Candida albicans* *Escherichia coli*

Figure 5. Antimicrobial activity of different crude extracts of *Ocimum basilicum* L.

1 = PE
2 = H_2O
3 = EtOAc
4 = EtOH

Table 6. Antimicrobial Activity of Crude Extracts of *Ocimum basilicum* L. (Leaves) on Six Species of Microorganisms

		Inhibition Zone Diameters (mm)					
Sample	Extracts	*B. subtilis*	*S. aurents*	*P. aeruginosa*	*B. pumilus*	*C. albicans*	*E. coli*
Thai Pin Sein (leaves)	PE	-	-	-	-	-	-
	EtOAc	11	11	-	-	12	12
	EtOH	13	14	12	12	13	13
	H_2O	-	-	-	-	-	-
Control	PE	-	-	-	-	-	-
	EtOAc	-	-	-	-	-	-
	EtOH	-	-	-	-	-	-
	H_2O	-	-	-	-	-	-

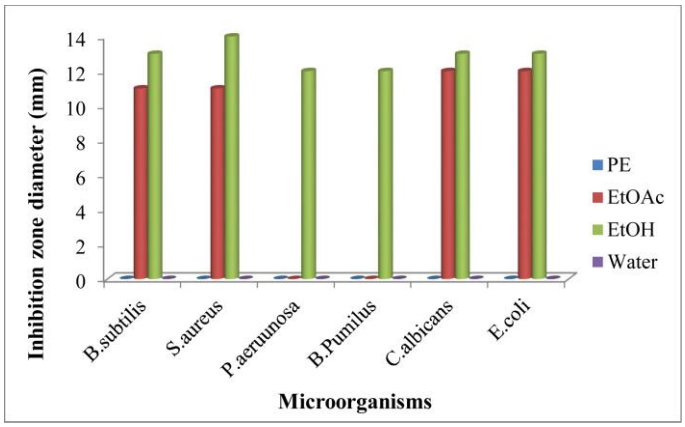


Figure 6. Antimicrobial inhibition zone diameters of crude extracts of *O*cimum *basilicum* L. (Thai Pin Sein)

Conclusion

Determination of nutritional values of *Ocimum basilicum* L. have been also carried out by AOAC method, resulting 12.56 % of moisture, 12.79 % of ash, 3.72 % of fat. 10.54 % of fiber, 27.01 % of protein, 33.38 % of carbohydrate, and 275.04 kcal/100g of energy values.

Total phenolic content was determined by FCR reagent method. The total phenolic content of EtOH extract 401.01 µg GAE/ mg and watery extract 573.63 µg GAE/ mg was observed. The total phenolic content of watery extract was higher than that of ethanol extract.

The total flavonoid contents were determined by spectrophotometric method using aluminium chloride reagent. The total flavonoid content of ethanol extract 26.85 µg QE/mg and watery 74.07 µg QE/mg was observed. The total flavonoid content of watery extract was higher than that of ethanol extract. Among two extracts, the watery extract of *O. basilicum* L. contained higher concentration of phenol and flavonoid contents than that of ethanol extracts.

O. basilicum L. was found to have radical scavenging activity. IC_{50} values of ethanol and water extracts are 9.68 and 8.71 µg/mL respectively. Water extract of *O. basilicum* L. has higher antioxidant activity than ethanol extract. All extracts showed slightly lower antioxidant activity when compared to the standard BHT (IC_{50} = 6.85 µg/mL).

The antimicrobial activities of polar and non-polar extracts such as pet-ether, ethyl acetate, ethanol and water extracts were tested on six microorganisms by agar well diffusion of tested microorganisms with the inhibition zone diameters in the range method. Among the tested extracts, EtOH extract of *O. basilicum* L. (leaves) inhibited all species between 11−14 mm. However, EtOAc extracts inhibited four species of microoraganisms namely *Bacillus subtilis, Staphylococus aureus, Candida albicans and Escherichia coli.* PE and H_2O extracts of *O. basilicum* L. do not show antimicrobial activity on six tested microorganisms.

Acknowledgements

The authors would like to thank Ministry of Education, Myanmar, for giving us the opportunity to present this research paper. We also would like to thank to the Department of Higher Education (Lower Myanmar), Ministry of Education, Myanmar for providing us the research facilities during this research.

References

AOAC. (2000). Official Methods of Analysis, Association of Official Analytical Chemists. Washington D. C: 17 th Ed., pp.526.

Haque, A.M., Nahar, N. and S.I. Khan, S.I. (2005). "Isolation of bioactive Secondary Metabolited from the Endophytic Engue of *Ocimum basilicum* L." *J. Dhaka Univ. J. Pharm. Sci.*, Vol.4 (2), pp.127-130.

Mamta, S., Sexena, J., Nema, R., Singh, D. and Gupta, A. (2013). "Phytochemistry of Medicinal Plants".
J. Pharm. and Phytochem, Vol.1 (6), pp.168-170.

Marinova, G. and Batchvarov, V. (2011). "Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH". *J. Agri. Sci.*, vol.17 (1), pp.11-24.

Priestman, J. and Edward, F.C.G. (1954). "Acid to PharmacecuticalAnalysis". *Ameri. Pharm. Asso.*, Vol.43 (2), pp. 65-128.

Poonkodl, K. (2016). "Chemical Composition of Essential Oil of *Ocimum basilicum* L. and its Biological Activities". *J. Critical Reviews*, Vol.3 (3), pp. 56-62

Rubab, E, Husain, I. and B .A. Khan, B.A. (2017) "Biomedical Description of *Ocimum basilicum* L." *J. IIMC.*, Vol. 12 (10), pp. 59-67.

Saxena, M., Saxena, J., Nema,R., Singh, D. and A. Gupta, A. (2013). "Phytochemistry of Medicinal Plants".
J. Pharm. Phytochem., Vol.1 (6), pp.168-182.