

Qualitative and Quantitative Secondary metabolites of *Anethum graveolens* L.

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Abstract:

The herbal products today symbolise safety in contrast to the synthetic that are regarded as unsafe to human and environment. Due to their safe, effective and inexpensive nature, indigenous remedies are popular among the people of both urban and rural areas in many countries. For present study an annual aromatic herb Dill (*Anethum graveolens* L.) was selected which belong to Apiaceae family. It has been cultivated since ancient times. Phytochemical evaluation is one of the tools for the quality evaluation of plants, which avails us with the prospect to see at a glance about the various phytochemicals present in a plant material. The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening. Preliminary phytochemical screening of different solvent extracts of *Anethum graveolens* L. showed the presence of many types of chemical constituents such as alkaloids, flavonoids, terpenoids, saponin, steroids, tannins. During present study, it was observed that leaves contain highest amount of alkaloids in D.W. as compare to the other solvent used.

Key words: *Anethum graveolens* L., Alkaloids, Flavonoids, Terpenoids, Saponin, Steroids, Tannins.

Introduction:

The herbal products today symbolise safety in contrast to the synthetic that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance. India is a well known country for various types of medicinal plants and leading in spices and medicinal plants in the world (Farooqi *et al.*, 2001). Some medicinal plants have great pharmaceutical value and have been traditionally used as home based remedies (Prajapati *et al.*, 2005). For present study an annual aromatic herb Dill (*Anethum graveolens* L.) was selected which belong to Apiaceae family. It has been cultivated as a vegetable, a carminative, an aromatic and antispasmodic plant. (Hornok, 1992 and Sharma, 2004). Numerous pharmaceutical industries play an important role in the identification of crude drugs derived from plant (Savithamma *et al.*, 2011). Phytochemical evaluation is one of the tools for the quality evaluation of plants, which avails us with the prospect to see at a glance about the various phytochemicals present in a plant material (Sofowora, 1986). In order to find out new bioactive compounds, extracts are concurrently evaluated by chemical screening (Wing, 1999). *Anethum graveolens* L. produces of secondary metabolites. Quantitative analysis of alkaloids was done

for leaf. Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties.

Material and method:

Dill (*Anethum graveolens* L.) plants were grown and maintained in the Department of Botany, Gujarat University, Ahmedabad. Plants were grown (5.6 pH) in sandy loam soil with 42% to 45% humidity. Temperature was maintained at 30°C to 35°C and plants were irrigated at every 3 days intervals.

Plant extracts preparation: The whole plant was dried at room temperature for 10 days including flowers, leaves, stems, and shoot. The dried plant material was milled and grounded into fine power. 10 gm of dried plant material was taken in 100 ml solvents and was kept for 12-16 hours. The extract was then filtered by Whatman filter paper (Harborne, 2005). The extract was collected and residue was dried overnight. And then the residue was then kept into the next solution for 12-16 hours and again the whole procedure was repeated. The *in vivo* plant extracts were prepared from roots, stems, leaves, seeds, while the *in vitro* extract were prepared from callus. Each sample was then transferred to glass vials and kept in refrigerator at 4°C for their future use in phytochemical analysis. Extracts for Root, Stem, Leaf and Seed were prepared in different solvents *i.e.* Petroleum ether, Methanol, Acetone, Ethanol, Chloroform, Distilled water.

Qualitative analysis: Standard protocols for Alkaloids and Saponins (Harborne, 2005), Flavonoids (Evans and Trease 2002), Terpenoids (Sofowora, 1993), Steroids (Brain and Turner, 1975), Tannins (Kokate *et al.*, 2009) were used for qualitative analysis of plant extracts.

Quantitative analysis for total alkaloids: Quantitative analysis for total alkaloids was done for leaf extract derived from different solvents. 5 gm of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and covered and allowed to stand for 4 hrs. This was filtered and extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residues of the alkaloids were dried and weighted (Harborne, 1973).

Statistical analysis

All the measurements were carried out in triplicate and the results were presented as mean values \pm SD. Statistical analyses were performed using a one- way analysis of variance ANOVA test and significance of the difference between means was determined by Duncan's multiple range test. Differences at $P \leq 0.05$ were considered statistically significant. The SPSS 20.0 (Chicago, Illinois, USA) was used to perform statistical analysis.

Result and discussion:

Anethum graveolens L. (Apiaceae), commonly known in English as ‘dill’ and in Hindi as ‘Soya’, is an annual herb found in Mediterranean region, Europe, and Central and South Asia. *A. graveolens* is used as an aromatic herb as well as medicine due to its high therapeutic value (Bahramikia *et al.*, 2009). It is used in Iranian folk medicine as an antihypercholesterolaemic antihypercholesterolaemic plant (Bahramikia *et al.*, 2008). It has traditionally been used for gastrointestinal ailments (Radulescu *et al.*, 2010). It is well known for its pharmacological effects, such as anti-microbial (Kaur *et al.*, 2010; Kazemi *et al.*, 2012; Albayrak *et al.*, 2012), antioxidant (Shyu *et al.*, 2009), anti-spasmodic, antisecretory, and mucosal protective effects (Radulescu *et al.*, 2010). It also has significant lipid lowering effect being a promising cardioprotective agent (Shyu *et al.*, 2009). Various different compounds have also been isolated from its seeds, leaves, inflorescence and fruits (Santos *et al.*, 2002). The presence of flavonoids, phenolic compounds and essential oil in the seeds of *A. graveolens* make it an important component for the preparation of gripe water (Heamalatha *et al.*, 2011). The present study was carried out on the *Anethum graveolens* L. revealed that the presence of active phytochemical constituents. The phytochemical active compounds of Dill were qualitative and quantitative analysed from root, stem, leaves and seeds are separately.

Qualitative analysis:

Preliminary analysis of phytochemicals is essential for the quantitative estimation of bioactive chemical compounds. (Sharanabasappa *et al.*, 2007). Preliminary phytochemical screening of different solvent extracts of *Anethum graveolens* L. showed the presence of many types of chemical constituents such as alkaloids, flavonoids, terpenoids, saponin, steroids, tannins, as shown in Table-1.

Table-1: Showing presence of phytochemical compound in root, stem, leaf and seed from different solvent.

Phytochemical Compound	Plant Part	Solvent					
		Distilled Water	Methanol	Ethanol	Petroleum ether	Chloroform	Acetone
Alkaloids	Root	+	-	-	+	+	+
	Stem	+	+	+	+	+	+
	Leaf	+	+	+	+	+	+
	Seed	+	+	+	+	+	+
Flavonoids	Root	+	-	-	-	-	-
	Stem	+	-	-	+	+	-
	Leaf	+	-	-	+	+	-

	Seed	-	+	+	-	-	+
Terpenoids	Root	+	-	+	+	+	-
	Stem	+	+	+	+	+	-
	Leaf	-	-	-	+	-	-
	Seed	+	+	+	+	+	+
Saponins	Root	-	-	-	+	-	-
	Stem	+	-	-	+	+	+
	Leaf	+	-	-	+	+	-
	Seed	+	-	-	+	+	-
Steroids	Root	-	+	+	+	+	+
	Stem	-	+	+	+	+	+
	Leaf	+	+	+	+	+	+
	Seed	+	+	+	+	+	+
Tannins	Root	+	+	+	+	+	+
	Stem	+	+	+	+	+	+
	Leaf	+	+	+	-	+	+
	Seed	+	+	+	+	+	+

The ethanolic and D.W. extracts from different parts of *Anethum graveolens L.* shows the presence of maximum phytochemicals when compared to other solvent extracts inferring the nature of solubility of the phytochemicals in polar solvents. It is evident from the results of the preliminary phytochemical analysis that alkaloids, flavonoids, terpenoids, saponins, steroids and tannins are present in the ethanolic extracts of roots, stem, leaf and seed. The present study shows that significant variation in the contents like alkaloids, saponins, flavonoids, tannins, terpenoids and steroids. The variations are due to number of environmental factors such as climate, altitude, rainfall etc. as mentioned (Kokate *et al.*, 2004).

Quantitative Assay of Total Alkaloids

Alkaloids are among the most active secondary metabolites and widely distributed in the plant kingdom (especially in angiosperms). Their structures contain one or several nitrogen atoms either in a ring structure (true alkaloids) or in a side chain (pseudoalkaloids). Depending on the ring structures, alkaloids are subdivided into several subgroups (Dewick 2001; Roberts and Wink, 1998).

During present study, it was observed that leaves contain highest amount of alkaloids in D.W. followed by the Acetone, Petrollium ether, Methanol, Ethanol and Chloroform. According to Lamkaden *et al.*, 2001 alkaloids have been shown to exhibit cytotoxic effect on tumour cell lines emphasizing its role in prevention

of cancer, neurodegenerative diseases, chronic inflammation etc. Alkaloids are infamous as animal toxins and certainly serve mainly as defense chemicals against predators (herbivores, carnivores) and to a lesser degree against bacteria, fungi and viruses. As discussed above, the molecular targets of alkaloids and amines often are neuroreceptors, or they modulate other steps in neuronal signal transduction, including ion channels or enzymes, which take up or metabolize neurotransmitters or second messengers (Van Wyk and Wink, 2004; Van Wyk and Wink, 2015).

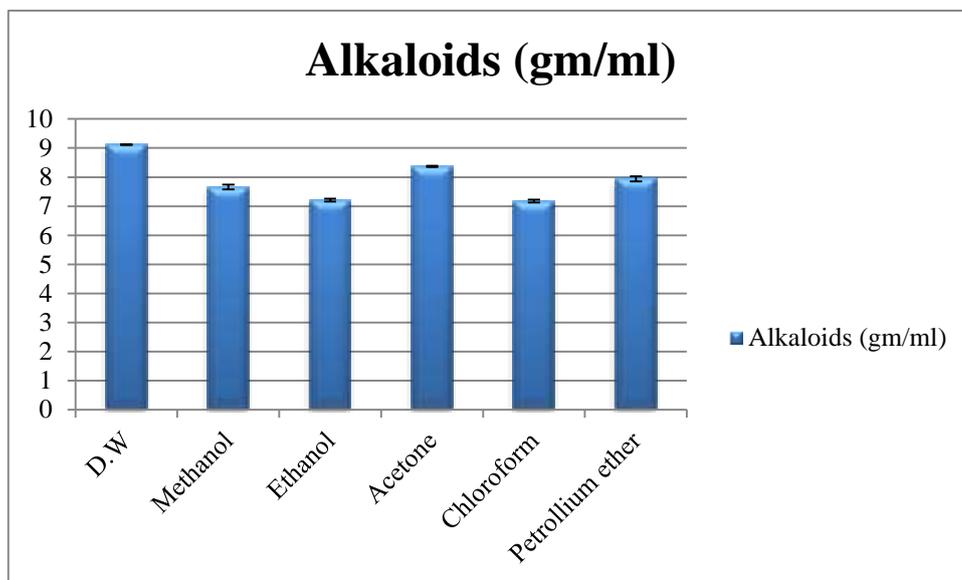


Figure-1: Quantitative analysis of Alkaloids from leaf in different solvent.

Conclusion: Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development, referred as secondary metabolites. Phytochemical screening of different solvent extracts of *Anethum graveolens* L. showed the presence of chemical constituents such as alkaloids, flavonoids, terpenoids, saponin, steroids, tannins. During present study, it was observed that leaves contain highest amount of alkaloids in D.W. as compare to other solvents.

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