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Quantitative Estimation of Phytochemicals from Essential Oils

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ABSTARCT

The aim of the present study was to evaluate the phenolic, flavonoid, terpenoid, aldehyde content, etc from essential oils. The oil was comprised to phytochemical screening and quantitative determination by spectrometric methods. Total phenolic content and total flavonoid content were evaluated according to the Folin-Ciocalteu procedure, and a spectroscopic method, respectively.

Keywords: Phenolic, Flavonoid, Rosemary, Lemongrass.

INTRODUCTION

Essential oils are volatile mixtures of secondary metabolites. They are also referred to as 'volatile oils'. The phrase essential oil was coined in the 16th century by Paracelsus von Hohenheim, who referred to the active ingredient in medication as Quinta essential (Guenther et., al. 1948). The components of essential oils include monoterpenes, sesquiterpenes, their derivatives (mainly oxygenated), diterpenes, aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters, lactones, nitrogen- and sulfur-containing chemicals, coumarins, and phenylpropanoids (Sabulal et., al. 2009). Plant essential oils are widely utilized in medicine, fragrances, cosmetics, bath treatments, and food preservatives, and the international market trades more than 250 types of essential oil-based goods worth $1.2 billion each year (Pandey et al., 2017; Swamy et al., 2016).

The chemical ingredients of plant essential oils are divided into two groups: terpenoids and phenylpropanoids. Terpenoids have a wide range of oxygenated derivatives, including alcohols, esters, aldehydes, ketones, ethers, peroxides, and phenols, as well as diverse carbon skeletons. The molecular weights of terpenes and their oxygenated derivatives differ only

slightly. The most important essential oil ingredients include hydrocarbon and oxygenated derivatives of monoterpenes, sesquiterpenes, and phenylpropanoids. Other chemicals present in essential oils include diterpenes, sulfur- and nitrogen-containing components, and lactones. Essential oils from different plants have gained much interest due to their antioxidant, antitumor, antibacterial, antifungal, and insecticidal properties (Burt, et.,al. 2004).

PLANT USED

Rosemary

Biological source- *Rosmarinus officinalis* commonly known as rosemary is a shrub with fragrant, evergreen, family Lamiaceae.

Chemical Constituents

The essential oil content from the leaves of R. officinalis was 0.93 g 100g-1. The major components determined in R. officinalis essential oil were 1,8-cineol (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%).

Fresh Lemongrass:

Biological source- *Cymbopogon citratus*, Belonging to family: Poaceae.

The major components in most lemongrass species include neral, isoneral, geranial, isogeranial, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol that make up about 60–80%.

MATERIAL METHODS

Collection of Plant Material and Extraction of Essential Oils

Fresh, air-dried Rosemary and Lemongrass samples were collected from the garden and authenticated in the botanical department by a botanist. Rosemary (RO oil) and Lemongrass (CC oil) essential oil isolated by steam distillation. All the samples were stored in glass vials. Essential oils were collected after decantation and tested for phenolic, flavanoid, and aldehyde content.

Total phenolic content

In the plant kingdom, phenols, aromatic molecules with hydroxyl groups, are abundant. They can be found in plant components. Plants that contain phenols are thought to be resistant to diseases and pests. Tannins, flavonols, and other phenolic chemicals are examples of phenols. The Folin-Ciocalteau reagent can be used to calculate total phenol. Formulation's total

phenolic content was determined using the Folin-phenol reagent method. Using a UV spectrophotometer, the blue color created by the polyphenol contained in the extract was quantified at 760 nm and expressed as mg/gm of Gallic acid equivalent (GAE).

The procedure of estimation of total phenolic content was determined as per the method given in (Kaur and Kapoor 2002). In brief, the oil extracts (0.1 ml)was mixed with Folin-ciocalteu phenol reagent (0.2ml), water (2 ml), and sodium carbonate (15%w/v; 1ml), and absorbance was measured at 660 nm using a spectrophotometer (Agilent Cary 60 UV) after 2hrs incubation period at 50^{o}C for 10 min. The experiment was performed in triplicates. The total phenolic content is expressed as µg per gm gallic acid equivalents.

Total flavonoid content

The procedure of estimation of total phenolic content was determined as per the method given in (Khadabadi et.al; 2019). In brief, Sample solution (0.5 ml), ethanol (1.5 ml), Al (NO3)3 (0.1 ml, 10%), CH3COONa (0.1 ml, 1 M), and water (2.8 ml) were thoroughly mixed and kept at ambient temperature for 40 min. The absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (Agilent Cary 60 UV). All the experiment was performed in triplicate. Total flavonoid content was calculated according to a standard calibration curve and expressed as mg per gm of quercetin equivalents (QE).

Total terpenoid content

The total terpenoid content (TTC) of the oil of Rosemary and Lemongrass was determined by the method of Ghorai et al. (2012). To 1 ml of the oil, we added 2 ml of chloroform. The sample mixture was then vortexed thoroughly before being left for 3 min. Subsequently, 200 µl of concentrated sulfuric acid (H_2SO_4) was poured into the mixture, followed by incubation at room temperature for 1.5–2 hr in the dark. A reddish-brown precipitate was formed in the mixture during incubation. After that, the supernatant was carefully decanted without disturbing the precipitation, and 3 ml of absolute methanol was added and vortexed well until the complete dissolving of the precipitation in methanol. Absorbance was read at 538 nm using a visible spectrometer. The TTC of the oil was calculated as mg of linalool per gram of extract. The equation of the standard curve was $y = 0.0036x - 0.001$, where $R2 = 0.9927$

Total aldehyde content

Reagent

1. Hydroxylamine hydrochloride – 3.47gm in 95ml of 60% alcohol
2. 0.5 N potassium hydroxide in 60% alcohol and make up the volume 100ml

3. Methyl orange – 0.5 ml 0f 0.2% w/v in 60% alcohol.

Procedure

Weigh accurately about 10g of oil in a stoppered tube. Add to it, 7ml of hydroxylamine hydrochloride reagent in alcohol and a drop of a solution of methyl orange. Titrate the liberated acid with 0.5 N potassium hydroxide in alcohol until the red colour change to permanent yellow in the lower layer. Calculate the aldehyde content as follow: 1 ml of 0.5 N potassium hydroxide in alcohol is equivalent to 0.07672 of citral (C.K.Kokate et.,al.)

RESULT AND DISCUSSION

Total phenolic content

The biological activity of polyphenolic compounds may be related to the chelation of metals, inhibition of lipoxygenase, and scavenging of free radicals (Mizuno T. et. al. 2002). he polyphenolic compounds contain hydroxyl groups in their structure and electron donating ability, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers and are responsible for antioxidant property (Javanmardi, et al.,2003). The highest total phenolic was recorded in RO oil contain 19.82 ±0.01 and CC oil contain 22.64 ± 0.01 (Figure -1)

Total flavonoids content

Flavonoids are the plant pigments. It is giving colour to flowers. They are ketone containing compounds. The antioxidant component flavonoid induces antiinflammatory activity. It inhibits the reactive oxygen compounds and the pro inflammatory activity of the enzyme cyclooxygenase. Flavonoids have potent anti-inflammatory activities by inhibiting prostaglandin synthesis (Lee D.Y. et.,al. 2007). The amount of total flavonoid content was determined using of standard compound quercetin (finger 7.2). The total flavonoid content in RO oil was 18.34 ± 0.02 , While CC oil show highest flavonoid content 28.56 ± 0.01. (Figure -2)

Total terpenoids content

Terpenoids are natural secondary metabolites found in plant species that provide flavour and fragrance. It prevent the development of chronic joint swelling (Agnihotri.S. et.,al. 2010). Linalool is a monoterpene that produces geraniol upon reaction with chloroform (Pushker A.k. et.,al. 2011). The reaction gives a characteristic reddish brown the concentration of

which shall be detected by absorbance at 538 nm. The interpretation of the results from the standard curve (Figure- 3) showed that the amount of terpenoid present in the RO Oil for 100µl/ml is 59.3 ± 0.02 and CC oil for 100µl/ml is 42.75 ± 0.02.(table 7.1). Terpenoids are reported to have antiinflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and antibacterial activity (Wang G. et.,al 2005).

Total aldehyde content

The higher aldehyde content in RO oil (about 11.26 ± 0.23) in CC oil aldehyde content (7.03 ± 0.4). However, the higher values of the aldehyde in the commercial samples (3 to 5%). In this method, the ketones also take part in the reactions, but their contribution is negligible since the RO oil and CC oil are essentially rich in aldehydes type of terpenoids.

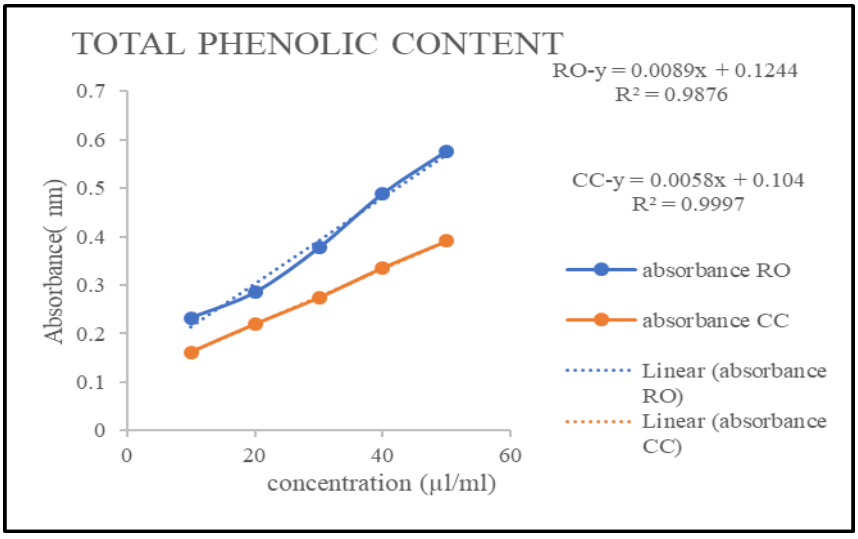

Fig.1: Phenolic content estimation

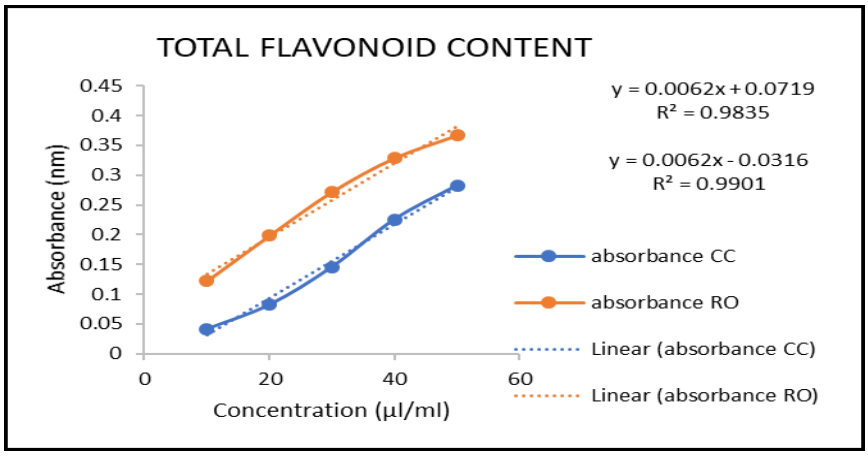

Fig. 2: Flavonoid content estimation

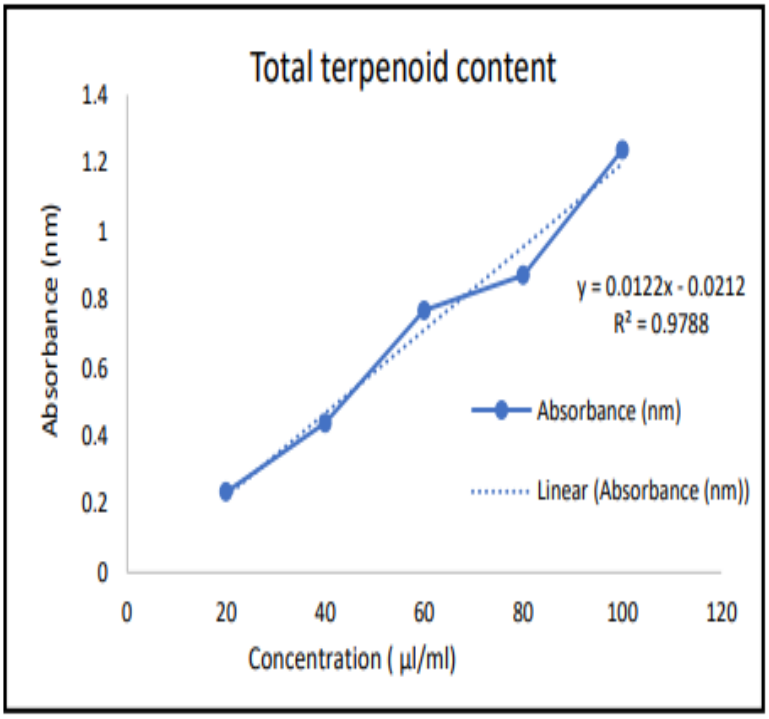

Fig. 3: Terpenoid content estimation

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