# QUALTITATIVE AND QUANTITATIVE ESTIMATION OF TERPENOID CONTENTS IN SOME IMPORTANT PLANTS OF PUNJAB, PAKISTAN

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**ABSTRACT:** Terpenoid contents of traditionally used ethnobotanically important plants (n=25) were determined. Ethanol extracts of seeds, fruits, pods, branches, bark, rhizome and whole plant parts were prepared and preliminary phytochemical investigation was carried out. Total terpenoid contents in ethanol extracts were determined through qualitative and quantitative analytical assay by Salkowski test. Results of qualitative analysis revealed that the seeds, pods and fruits were rich in terpenoid contents. Whereas the quantitative analysis of ethanol extract showed that fruit of *Carissa carandas* and *Lodoicea maldivica* seeds of *Erysimum cheiri*, whole plant of *Fagonia arabica* and branches of *Withania somnifera* had 80% of total terpenoids contents. While, seeds of *Ceiba speciosa* contained 77%, rhizome of *Berberis lyceum*, flower of *Rosa indica* and fruit of *Diospyros peregrina* had 70% of total terpenoids contents. Terpenoid contents in all other selected plants ranged from 60 to 40%. This study would be helpful in the determination of therapeutic bioactive terpenoids that could be isolated and used for mankind welfare.

**Key word:** Terpenoids, Therapeutic use, Salkowski test, Terpenoids estimation.

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## INTRODUCTION

Plants produce a large numbers of secondary metabolites as bioactive compounds such as alkaloids, tannins, flavonoids, sterols and terpenes, etc., that have a major role in nutrition, physiology and control of diseases. Terpenoids are one of the most important classes of bioactive compounds in higher plants, hence the foremost task is the screening of these compounds in the plants. According to world health organization, 80% of the world's population rely upon such traditional plant-based systems of medicine to provide them with primary healthcare (WHO, 2004). The use of bioactive compounds as herbal medicines continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings (Ekor, 2013).

*D.malabarica* (Ebenaceae) contains antimicrobial, antioxidant and blood purifying compounds (Ravikumar *et al*., 2014). *F.arabica* (Zygophyllaceae) plant is used as blood purifier, anticancer, neuroprotective and is a source of fever remedy (Jayat *et al*., 2007). *B.lycium* (Berberidiaceae) plant has antispasmodic, antioxidant, antimicrobial properties and is used for treatment of Jaundice (Mashwani *et al*., 2013). *R. Indica* (Rosaceae) is medicinal and contains cardio-tonic, diuretic, expectorant, antiviral antioxidant, antibacterial, mutagenic/anti-mutagenic, anti-cholinesterase, antityrosinase and anti-diabetic properties (Zengin *et al*., 2014). *F. lyrata* contains antioxidant, antimicrobial and anticancer properties (Bidarigh *et al*., 2011). *H. adenophyllum* (Bignoniaceae) is used for various ailments treatment which includes cancer, gastrointestinal disorders, cholera, rheumatoid arthritis, hepatic disorders, leucorrhea and diabetes (Rahmatullah *et al*., 2010). *C. paniculatus* (Celastraceae) is used in the treatment of cancer, cognitive dysfunction, epilepsy, insomnia, rheumatism, gout and dyspepsia (Lekha *et al*., 2010).

*S. potatorum* (Loganiaceae) is used for the treatment of gonorrhea, leukeorrhea, gastropathy, bronchitis, chronic diarrhea, dysentery, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, ulcers, and other eye disease (Yadav *et al*., 2014). *C. behen* (Asteraceae) plant is used in the treatment of cancer and neurological disorders (Esmaeili *et al*., 2013). *D. peregrina* (Ebenaceae) has antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, cathartic, expectorant and anticancer (Dewanjee *et al*., 2007). *C. carandas* (Apocynaceae) is rich in iron, vitamin C and pectin hence is used in the treatment of anemia, oxidative stress and preparation of jelly, jam and syrup (Shafeeq *et al*., 2014). *C. verum* (Lauraceae) has strong antioxidant, antibacterial, antipyretic and anti-inflammatory antitumor and anticancer compounds (Hamidpour *et al*., 2015).

Epidemiological, experimental and research studies suggest that terpenoids are helpful in the

prevention and therapy of several cancer diseases, including mammary, skin, lung, fore-stomach, colon, pancreatic and prostate carcinomas. A large number of triterpenoids suppress the growth of a variety of cancer cells without exerting any toxicity in normal cells. Numerous preclinical efficacy studies have provided extensive evidence that both naturally occurring and synthetic derivatives of triterpenoids possess chemopreventive as well as therapeutic effects against colon, breast, prostate and skin cancer (Sakarkar and Deshmukah, 2011 and Carro *et al*., 2013).

The objective of the present study was to investigate the therapeutic importance of terpenoid contents as bioactive compounds.

## MATERIALS AND METHODS

**Collection of Plants:** Fresh fruits/seeds/pods/rhizome/flowers and bark of plants were collected from different locations in the month from Jan-March (2016). The voucher specimens were deposited in the herbarium of the Lahore College for Women University. *Prosopis Juliflora* (Sw.) DC. (LCWU-15-128), *Carissa carandas* L. (LCWU-15-129), *Ceiba speciosa* A. St. Hil. Ravenna (LCWU-15-130), *Heterophragma adenophyllum* Wall. (LCWU-15-131), *Cestrum diurnum* L. (LCWU-15-132), *Jacaranda mimosifolia* D. (LCWU-15-133), *Diospyros malabarica* (desr.) kostel. (LCWU-15-134), *Terminalia bellerica* Roxb. (LCWU-15-135), *Ficus lyrata* L. (LCWU-15-117), *Diospyros peregrina* fo. javanica Kosterm. (LCWU-15-136), *Cinnamomum verum* J. (LCWU-15-137), *Erysimum cheiri* L. (LCWU-15-138), Buchanania lanzan Spreng. (LCWU-15-139), *Withania somnifera* L. (LCWU-15-89), *Fagonia arabica* L. (LCWU-15-140), *Berberis lycium* Royle. (LCWU-15-141), *Strychnos potatorum* L.f. (LCWU-15-142), *Matthiola incana* (L.) W.T.Aiton. (LCWU-15-143), *Ziziphora tenuior* L. (LCWU-15-144), *Centaurea behen* L. (LCWU-15-145), *Rosa indica* L. (LCWU-15-122), *Punica granatum* L. (LCWU-15-146), *Lodoicea maldivica* (Pers.ex H. Wendl.) (LCWU-15-147), *Cassia absus* L. (LCWU-15-148), *Celastrus paniculatus* Willd. (LCWU-15-149). The fruits after washing were shade dried at room temperature (20°C-30°C). The dried plants were pulverized into fine powder and stored for the future use.

**Preparation of Plant Extracts:** The ground plant material was extracted with ethanol by maceration with continuous stirring for seven days. The resulting crude extract was filtered by passing through a Wattman no. 3 filtered paper followed by concentration in vacuum 40°C using a rotary evaporator (Malik *et al*., 2015). Percentage yield (w/w) of dried extracts is presented in Fig.1.

**Preliminary phytochemical assay:** Freshly prepared extracts were subjected to standard methods of phytochemical analyses (Harborne, 1998) to detect the presence of constituents, viz. flavonoids, alkaloids, phenolics and glycosides.

**Screening of Terpenoids**

**Qualitative test for terpenoids (Salkowski test):** Dried extract (50mg) was taken and soaked in 5 mL of ethanol. Extract was mixed in 2 mL of chloroform. It was slightly warmed then cooled. 3 ml of concentrated $H_2SO_4$ was added slowly along the sides of test tubes. A radish brown colored precipitation was formed at the interface indicating the presence of terpenoids (Indumathi *et al*., 2014).

**Quantitative test for terpenoids:** Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hour (Indumathi *et al*., 2014). The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100). The results are presented in Fig-2.

**Statistical Analysis:** All the *in vitro* experimental data were presented as mean ± S.E. (M± standard error) of three parallel measurements and data were evaluated by SPSS Statistical software (one way), p – values <0.05 were regarded as significant followed by posthoc.

## RESULTS AND DISCUSSION

Preliminary phytochemical analysis indicated the presence of flavonoids, alkaloids, saponins, terpenoids, glycosides and phenolic compounds in extracts of fruits, pods, seeds and bark leading to the medicinal properties of these plants (Indumathi *et al*., 2014). Percentage yield of dried ethanolic extracts was calculated (Fig-1). The variation in yield of extracts was due to the solubility rates and reactivity of terpenoid and other bioactive compounds in ethanol at room temperature. Ethanol extraction of plant lead to highly oxygenated mostly polar bioactive compounds like triterpenes, triterpenoid and glycosides (Bhat, 2005).

Salkowiski test was performed for qualitative as well as quantitative estimation of ethanol extracts of plants. All ethanol extracts showed the different intensity of brown colour ranging from deep brown to light brown indicating the presence of mono-terpenes and sesquiterpenes (essential oils), di- and triterpenoids (gums and resins), Tetraterpenoids (carotenoids, xanthophylls and carotenoic acids) and polyterpenoid is the rubber (Sameeno, 2007; and Raaman, 2006). The fruit of *C. carandas* and *L. maldivica*, seeds of *E. cheiri*, whole plant of *F.arabica* and branches of *W. somnifera* showed deep brown colour. While, seeds of *C. speciosa*,

rhizome of *B. lyceum*, flower of *R. indica* showed brown and fruit of *D. peregrina* showed light brown color. Plant terpenoids served as antimicrobial, antioxidant, anticancer, neuro-protective and chemo-protective properties (Malik *et al*., 2012; Kashif *et al*., 2014; and Malik *et al*. 2015).

The results of quantitative estimate of total terpenoid contents in ethanolic extracts of plants are presented in Figure-2.

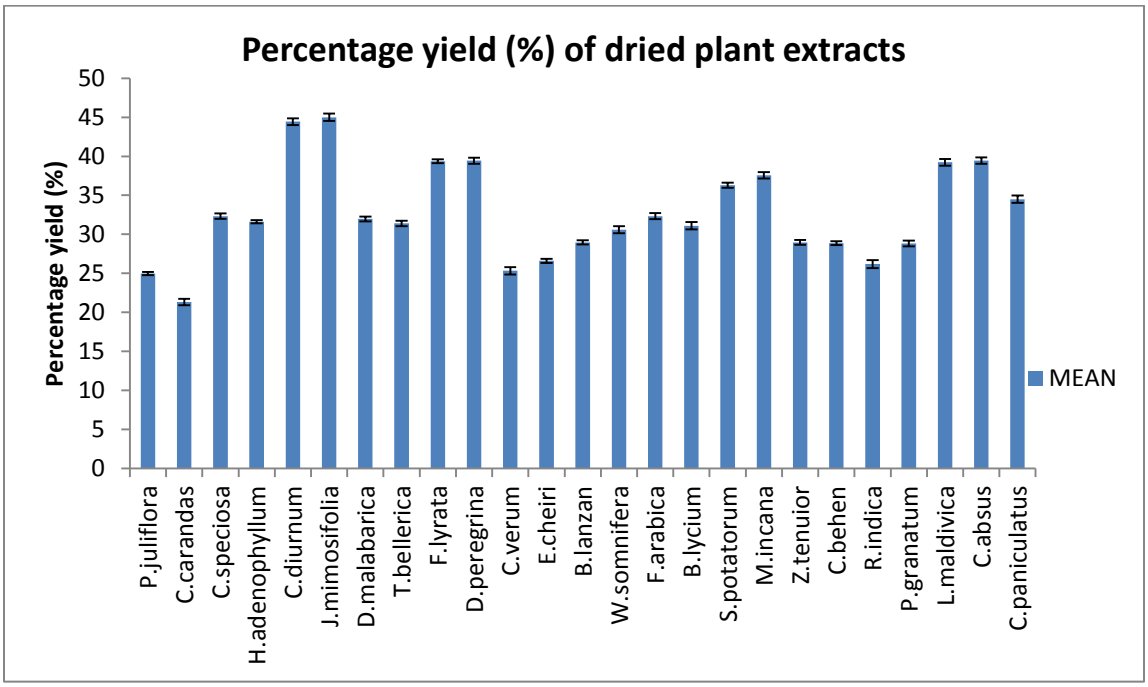


**Figure 1: Percentage yield of dried extracts of selected medicinal plants**

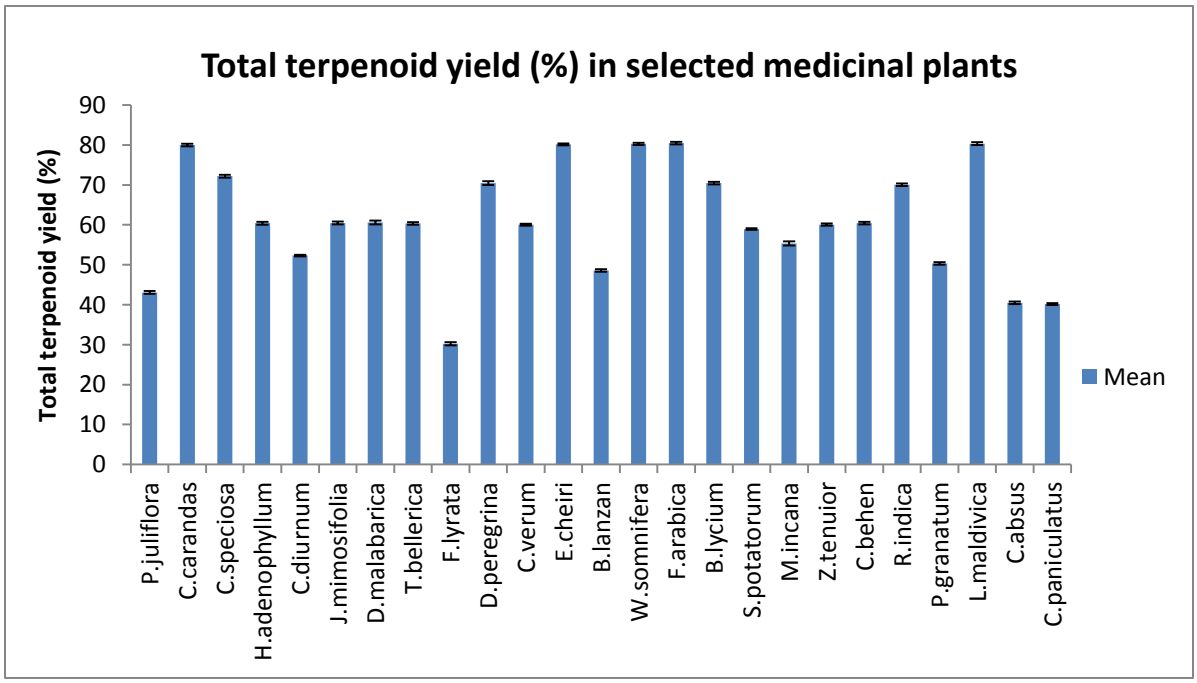


**Figure 2: Total terpenoid yield (%) of selected medicinal plants**

It was revealed from the results that that fruit of *C. carandas* and *L. maldivica*, seeds of *E. cheiri*, whole plant of *F. arabica* and branches of *W. somnifera* resulted into 80% of total terpenoids contents. While, seeds of *C. speciosa* showed 77%, rhizome of *B. lyceum* 70%, flower of *R. indica* 70%, and fruit of *D. peregrina* showed 70% of total terpenoids contents. All other selected plants gave a wide range of terpenoids from 60 to 40%. Terpenoids were the most widespread, chemically interesting groups of secondary metabolites with over 30,000 known compounds including steroids reported by (Wang *et al*. 2005; Umlauf, 2004, Ajaib *et al.*, 2016). Among the pharmaceuticals, the anticancer drug Taxol and the antimalarial drug Artimesinin were two of the most renowned terpene-based drugs (Wang *et al*., 2005, Raaman, 2006, Sameeno, 2007). Hence, the high percentage of total terpenoid contents in the fruits, seeds and bark of the selected trees endorse their use as a therapeutic medicine against deadly diseases like cancer and Alzheimer.

**Conclusion:** From the above applied workout, it can be clearly concluded that these plants contain high terpenoids. The above stated procedure is therefore an easy and simple approach to determine total terpenoid contents. Future research will be helpful in the isolation and separation of these terpenoids so that could be used for human welfare.

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