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EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF LAURUS NOBILIS EXTRACT

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ABSTRACT
Laurus nobilis L. (laurel) leaves are frequently used as a spice for cooking purposes. Folk medicine in many countries uses the infusion of the plant in stomachic and carminative remedies, as well as for the treatment of gastric diseases. Little information is available about the phytochemical composition of the infusion of dried leaves, which is a way to consume this aromatic and medicinal plant. Laurus nobilis L. (laurel) leaves are frequently used as a spice for cooking purposes. Folk medicine in many countries uses the infusion of the plant in stomachic and carminative remedies, as well as for the treatment of gastric diseases. Little information is available about the phytochemical composition of the infusion of dried leaves, which is a way to consumethis aromatic and medicinal plant. Laurus nobilis commonly known as bay leaf, sweet bay belonging to the family Lauraceae is one of the most useful industrial plant used in foods, drugs, cosmetics. The present study focused on the antioxidant activity of Laurus nobilis Leaf Extract in vitro conditions. The dried leaf of Laurus nobilis was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. All the analysis was made with the use of UV-Visible Spectrophotometer. In this plant Laurus nobilis Leaf Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. These findings demonstrated that Laurus nobilis Leaf Extract possess free radical and hydroxyl radical scavenging activity as well as antioxidant activity in vitro. In conclusion the present study indicates that L. nobilis Leaf Extract may be a potential source of natural antioxidant. The results suggested that L. nobilis Leaf Extract could serve as a potential source of antioxidant and can be used in any preparations for combating free radical mediated damage to the body.

KEYWORDS: Antioxidant activity, Fenton Reaction, Hydroxyl radical, ascorbic acid, Laurus nobilis, TBARS.

INTRODUCTION
Laurus nobilis is (Family Lauraceae), commonly known as bay, sweet bay, true laurel, or Roman laurel, is an evergreen tree widely distributed in the Mediterraneanarea and Europe. In folk medicine it is used for stomachic and carminative remedies as well as in the treatment ofgastric diseases (Afifi, et. al., 1997; Passalacqua, 2006). L. nobilis leaf extracts have been investigated for their wound healing, cytotoxic, and trypanocidalproperties (Kivcak, et al., 2002; Uchiyama, 2002; Dall'Acqua, et al., 2006; Nayak, 2006). Laurel has attracted continuous and renewed interest because of its pharmacological and health beneficial properties related to several compounds present in the plant. Previous phytochemical investigations on L. nobilis leaves and fruits led to the isolation of sesquiterpene lactones (Harborne, 1997), alkaloids (Pech, 1982), glycosylated flavonoids (Fiorini, 1998) and monoterpene andgermacrane alcohols (Novak, 1985; Appendino, 1992). However, little information only. nobilis infusion is thus far available because all the previously published data are in reference to organic solvent extracts. For this reason, we decided to investigate the infusion (IF) of dried laurel leaves. Previously published data are in reference to organic solvent extracts. For this reason, we decided to investigate the in-fusion (IF) of dried laurel leaves. India has a long history for ayurveda, Ayurvedic medicinal plant are used in traditional Ayurvedic medicine, which is based on plant have a preventing and treating of human diseases. Each plant or herb has a specific quality (Shakya, 2017). Medicinal plants have been recognized as potential drug candidates because they possess drug like properties. Laurus nobilis, belong to the Lauraceae family also known as laurel, sweet bay,

true laurel, Grecian laurel, Roman laurel, noble laurel, daphne, bay tree or laurel tree that have antioxidant properties (David and Alford 2012; Katherine and Schmid et al,. 1996) and used in foods, drugs, and cosmetics. It is have antimicrobial and insecticidal activity. Recently used in treating diabetes and preventing migraine (Ramling Patrakar et al., 2012). Therefore we have planned to carry out the antioxidant activity of Laurus nobilis Leaf Extract.

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MATERIALS AND METHODS

Plant material – Laurus nobilis Leaf was collected from Local Herbal Garden, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of Laurus nobilis Leaf (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of Laurus nobilis Leaf extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbance's were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \text{Abs: } \frac{\text{532 nm Control Abs. - 532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

RESULT

The result of the examined Laurus nobilis extract and control solution i. e Ascorbic acid shows that they inhibited the production of OH radicals. The percentage of free radical scavenging activity of the extract increases in concentration. Extent of hydroxyl radical scavenging was determine by the increase in intensity of light pink color which was determined at 532nm. The antioxidant activity was compared with ascorbic acid which was taken as appositive control.

Table 1: Percentage of Inhibition of ascorbic acid and Laurus nobilis sample.

SI	Concentration	OD of Ascorbic Acid (Mean ± SE)	OD of Laurus nobilis (Mean ± SE)
01	10	05.3±0.20	15.3±1.15
02	20	07.5±0.36	33.3±0.64
03	30	14.2±0.19	49.2±0.76

04	40	18.1±0.38	66.1±0.45
05	50	32.1±0.28	70.0±0.28
06	60	38.2± 0.35	72.6±0.38
07	70	63.7±0.47	75.5±0.35
08	80	67.4±0.54	77.0±0.48
09	90	75.6±0.53	80.2±0.32
10	100	79.3±0.31	82.1±0.44
Blank: 0.4320			

DISCUSSION

Previous studies on the antioxidant activity of L. nobilis leaf extract revealed a powerful activity for different extracts obtained with organic solvents.[26,27] But, to our knowledge no study has been reported on the L. nobilis IF that is one of the preparations used for consuming this medicinal and aromatic plant. Discrepancies between our present and previously published results could be related to the differences in solvents, extraction methods, and anti- oxidant tests used. In addition, the vegetal material com-

Discussion and Summary

Previous studies on the antioxidant activity of *L. nobilis* leaf extract revealed a powerful activity for different extracts obtained with organic solvents (Conforti, 2006; Kang, 2006). But, to our knowledge no study has been reported on the L. nobilis IF that is one of the preparations used for consuming this medicinal and aromatic plant. Discrepancies between ourpresent and previously published results could be related to the differences in solvents, extraction methods, and antioxidant tests used. In addition, the vegetal material composition is very often related to geographic, climatic, and environmental conditions, resulting in great variation of the secondary metabolite content.

Anti- oxidant activity of the isolated compounds measured with the BR method could be related to their chemical structures. In particular, the higher activity of the quercetin derivatives could be related to the catechol group, which is known to improve the antioxidant properties of the flavonol derivative.[25–32]

Moreover, except for compound 1, all the glycosides show an activity greater than or similar to that of the corresponding aglycone with the BR method. Quercetin derivative glycosides show a quite higher ac- tivity than the corresponding aglycone at acidic pH, while at pH 7.4 their activity is much lower than that of quercetin aglycone, as expected. For compounds 9and 10 the presence of the methoxy group in the ortho position with respect to the phenolic -OH may play a role in the antioxidant effect.[32]

Most of the compounds isolated from L. nobilis show good (i.e., compounds 9,10, and 13) an- tioxidant activity at either acidic and physiological pH. Obviously, we are aware that on no account can che-mical in vitro antioxidant assays mimic physiological conditions. On the other hand, recent in vivo experiments (on rats and mice) showed that several plant extracts with in vitro antioxidant activity (by the TEAC or BR Anti-oxidant activity of the isolated compounds measured with the BR method could be related to their chemical structures. In particular, the higher activity of the quercetin derivatives could be related to the catechol group, which is known to improve the antioxidant properties of the flavonolderivative. Moreover, except for compound 1, all the glycosides show an activity greater than or similar to that of the corresponding aglycone with the BR method. Quercetin derivative glycosides show a quite higher activity than the corresponding aglycone at acidic pH, whileat pH 7.4 their activity is much lower than that of quercetinaglycone, as expected. For compounds 9 and 10 the presence of the methoxy group in the ortho position with respect to the phenolic -OH may play a role in the antioxidant effect. 32Most of the compounds isolated from L. nobilis show good (i.e., compounds 9,10, and 13) antioxidant activity at either acidic and physiological pH. Obviously, we are aware that on no account can chemical in vitro antioxidant assays mimic physiological conditions (Conforti, 2006).

In conclusion, the study confirms that Laurus nobilis is a rich source of bioactive compounds with significant antioxidant properties. The phytochemical analysis revealed the presence of various phenolic, compounds, and flavonoids etc. which contribute to its biological activities. The antioxidant assays demonstrated that bay leaf extracts exhibit strong free radical abilities, supporting its traditional use as a natural preservative.

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