Antinociceptive, antioxidant and phytochemical studies of Pakistani medicinal plants

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Abstract: The aim of the current study was to evaluate the antinociceptive activity of the selected Pakistani medicinal plants (*Chenopodium botrys, Micromeria biflora* and *Teucrium stocksianum*) *in-vivo* followed by their antioxidant potential against 1,1-diphenyl-2-picrylhidrazyl (DPPH) *in-vitro.* The results demonstrated profound antinociceptive effect of both the crude methanolic extract of *Chenopodium botrys* (CBM) and subsequent aqueous fraction (CBW) of *C. botrys* with 80.76% and 84% pain relief in acetic acid induced writhing test at 100 mg/kg i.p respectively. Similarly the crude methanolic extract of *Micromeria biflora* (MBM) and its subsequent aqueous fraction (MBW) with 66.46% 78.08% pain reversal in acetic acid induced writhing test respectively at 100mg/kg i.p. However, the crude methanolic extract and isolated water fraction of *Teucrium stocksianum* (TS) did not show any significant effect at test doses. Both the crude extracts and aqueous fractions of selected medicinal plants exhibited marked scavenging effects on DPPH and therefore strongly support the antinociceptive activity. Phytochemical analysis indicated the presence of various classes of natural products (alkaloids, terpenoids, flavonoids etc.) and thus the current finding can be attributed to the presence of these compounds. In short, our findings provide a strong scientific background to the folk uses *C. botrys* and *M. biflora* in the management of various painful conditions.

Keywords: *Chenopodium botrys, Micromeria biflora, Teucrium stocksianum,* antinociceptive, antioxidant activity.

INDRODUCTION

Plant based products have a long lasting history in the treatment of various disorders including painful conditions (Calixto *et al*., 2000, Khan, 2014). The synthetic agents are used for the pain which are not providing such desirable clinical results and associate with several side effects (Calixto *et al.,* 2000). Focusing on natural products; which could be the best possible option for effective management of such conditions (Khan and Rauf, 2014). The current studies on the medicinal plants might be helpful for such treatment.

Chenopodium botrys L. is a member of Chenopodiaceae family which is known as Jerusalem oak. All parts of the plant have aromatic odors. In Iranian traditional medicines, the flowering aerial parts of *C. botrys* have long been used as expectorant, antiasthmatic, anticatarrh, anticonvulsant agent and as tonic. In many cases, *C. botrys* is used instead of lavenders to keep away moth. Chemical compositions of *C. botrys* essential oil have been reported by other researchers (Mahboubi *et al.,* 2013). Many species of *Chenopodium* are used for abdominal pain is traditional system (Yadav *et al.,* 2007). Various classes of chemical constituents such as flavonoids and betaines have reported from *C. botrys* (Rustembekova *et al.,* 1973; 1974).

Micromeria biflora Buch. Ham. ex D. Don belongs to family Lamiaceae, which is found in tropical, and Himalaya region. *M. biflora* was rubbed and the aroma inhaled to treat nose bleeds. A paste of the plant was used as a poultice to treat wounds and toothache analgesic (Hardie, 1992). The juice of the plant is taken internally and also inhaled in the treatment of sinusitis. *M. biflora* leaf essential oil has been widely used to construct bacterial phylogenetic relationships (Manandhar, 2002, Islam, 2007, Shukla, 2010). *M. biflora* roots and leaves are used for the cure of pain and ulcer (Bisht *et al.,* 2013).

Teucrium stocksianum Boiss subsp. *Stocksianum* belongs to family Labiatae. It has a dense compact herb, which grows mostly in the mountain. The leaves extract of *T. stocksianum* is used as a folk medicine for the treatment of diabetes mellitus and stomach diseases. *T. stocksianum aerial* parts contain various secondary metabolites such as saponins, terpenoids and flavonoids. *T. stocksianum* also possesses antimicrobial activity against a wide range of microbes (Wasfi *et al.,* 1995, Rasheed *et al.,* 1995). *T stocksianum* extracts poses gastric cytoprotective and hepatoprotective properties (Wasfi, 1992). *T. stocksianum*

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is traditional uses in the treatment of painful conditions (Radhakrishnan *et al.*, 2001).

The present paper is highlighted to evaluate the preliminary antinociceptive and antioxidant activity of three selected Pakistani medicinal plants, which are using traditionally for pain and related disorders.

MATERIALS AND METHODS

Plant collection

Chenopodium botrys, *Micromeria biflora* and *Teucrium stocksianum* were collected from the mountain area of Razagram Toormang, District Dir, Khyber Pakhtunkhwa province of Pakistan in the month of March, 2013. The plants were identified by Ghulam Jelani, Department of Botany, University of Peshawar, Pakistan. The voucher specimens No. U (PUP)-8825-8827 were deposited in the herbarium of the said department.

Extractions and fractionations

Shade dried plants of *C. botrys* (500g), *M. biflora* (500g) and *T. stocksianum* (500g) were extracted successively with aqueous and methanol (x3) at room temperature for 10 days. Dark-gray extracts were obtained by concentration under reduce pressure at 40^{0}C (Uddin *et al.*, 2011).

Acetic acid induced writhing test

BALB/c mice were used for determination of antinociceptive activity. The samples were screened using 50mg/kg and 100mg/kg doses i.p. and the antinociceptive effect was determined following our published methods (Khan *et al.*, 2011a, Barkatullah *et al.*, 2013).

Antioxidant bioassay

DPPH (1,1-diphenyl-2-picrylhidrazyl) bioassay was conducted to determine the antioxidant effect. This procedure was performed as according to standard protocol (Uddin *et al.*, 2012, Khan *et al.*, 2012b). The crude methanolic and aqueous extracts were screened for potential anti-radical activity on the basis of scavenging effects of the DPPH (1,1-diphenyl-2-picrylhidrazyl) (Waka. Ltd. Japan). The solution of DPPH was ready by dissolving DPPH (5mL) in the ethanol (2mL), keeping in dark at room temperature. Different concentrations of the

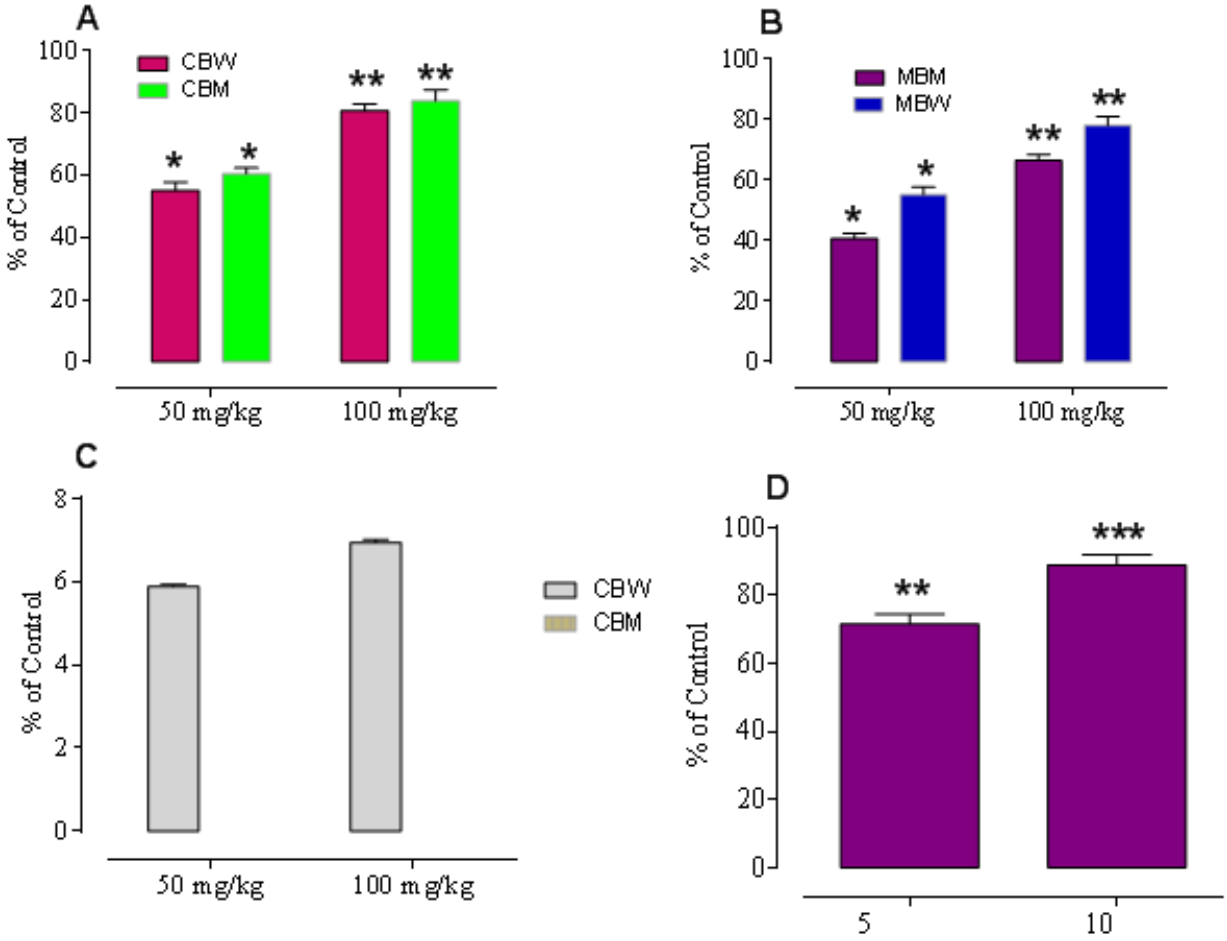

Fig. 1: The effect (%) of selected Pakistani medicinal plants in acetic acid induced writhing test. Values are reported as mean ± S.E.M. for a group of six animals in each case. The data were analyzed by ANOVA followed by Dunnett's test. Asterisks indicate statistically significant values from control. **P*<0.05, ***P*<0.01 or ****P*<0.001

crude extracts were prepared in ethanol and an aliquot was transfer to a 96 well micro-titer plate (Molecular Devices, USA). The combined reaction mixtures were incubated in Elisa at 37°C for 30min and the absorbance was measured at 517nm. Percentage inhibition of radical scavenging activity was determined by evaluation of the results with the control. The ascorbic acid was used as positive control while ethanol as negative control during the experiment. The analyses were done in triplicate. The % activity was calculated using the following formula:

$$\% \text{ DPPH} = \frac{\text{Control absorbance Extract absorbance}}{\text{Control absorbance}} \text{ X100}$$

Preliminary phytochemical tests

The phytochemical contents were determined using our previous published methods (Uddin *et al.,* 2011).

RESULTS

Effect of antinociceptive activity

The results of antinociceptive activity of the selected plants in acetic acid induced writhing test are shown in fig. 1 (A-C). As shown in fig. 1, *C. botrys* methanolic extract (CBM) and *C. botrys* water extract (CBW) demonstrated marked pain reduction in a dose dependent manner. The maximum inhibition was 80.76% and 84% in acetic acid induced pain model at 100mg/kg i.p respectively. In case of *M. biflora* methanolic extract (MBM) and *M. biflora* water extract (MBW), marked inhibition on noxious stimulation due to acetic acid induced pain was observed at test doses (50 and 100 mg/kg i.p.) in a dose dependent manner, MBM and MBW had maximum of 66.46% 78.08% pain reduction at 100 mg/kg i.p (fig. 1B). However, the methanolic and water extracts of *T. stocksianum* did not show any significant effects at test doses (fig. 1C). Nevertheless, standard used (diclofenac) exhibited more prominent antinociceptive action (fig. 1D).

Effect of DPPH free radical activity

The effects of selected plants in free radical scavenging assay are illustrated in fig. 2. The CBM and CBW showed significant scavenging effect on DPPH in a dose dependent manner (fig. 2A). Both CBM and CBW showed 91.97% and 90.33% activity at 100µg/ml respectively. When MBM and MBW were tested against DPPH, they showed marked quenching effect (fig. 2B). The maximum inhibition of MBM and MBW was observed as 71.01% and 80.31% at 100µg/ml, respectively. In case of TSM and TSW, profound effect was observed against DPPH in a concentration dependent manner (fig. 2C). At 100µg/ml, maximum inhibition of 95.26% and 98.04% was observed for TSM and TSW respectively.

Preliminary phytochemical analysis

The preliminary phytochemical analysis is presented in table 1. The results indicated the presence of various classes of natural products such as alkaloids, terpenoids, flavonoids, reducing sugars, soluble starch, combined reducing sugars and tannins to which the antinociceptive properly shown by plants could be attributed.

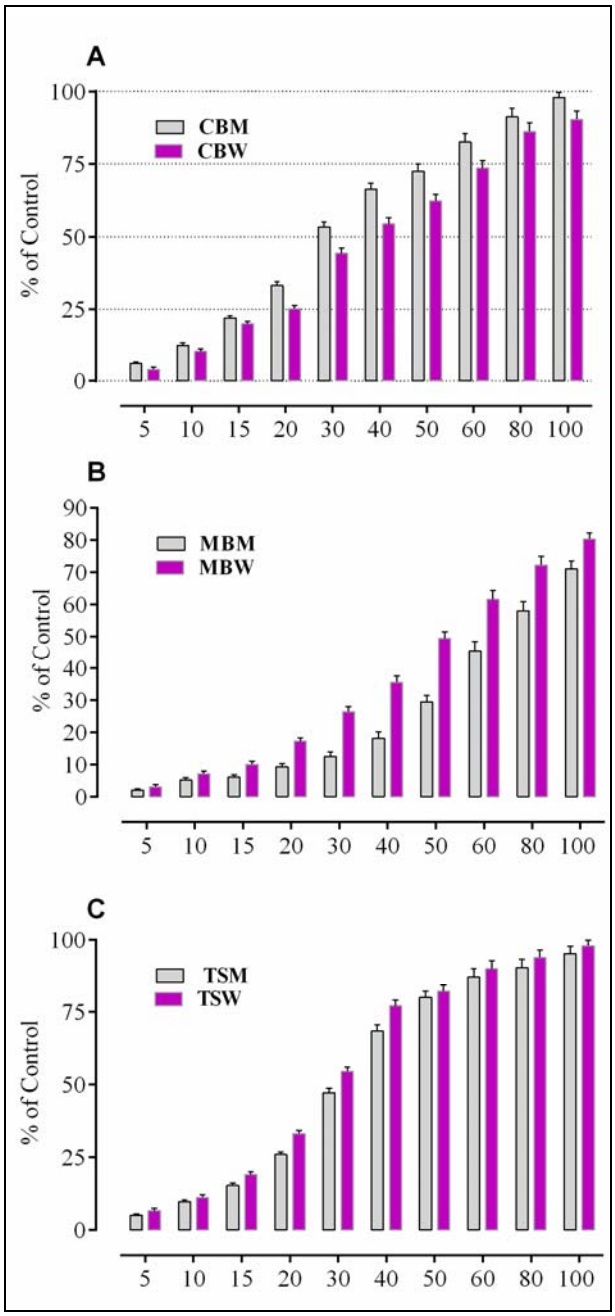

Fig. 2: The percent effect of tested Pakistani medicinal plants on DPPH at various concentrations. Values are mean ± SEM (n = 3).

DISCUSSION

In the present study, three selected Pakistani medicinal plants (*Chenopodium botrys*, *Micromeria biflora* and

Table 1: Phytochemical test of crude methanolic and water extract of *T. stocksianum, C. botrys,* and *M. biflora*

Constituents	TSW	TSM	CBW	CBM	MBW	MBM
Alkaloids	-	+	-	-	-	-
Steroids	-	-	-	+	-	-
Flavonoids	-	-	+	+	+	+
Tannins	+	+	+	-	+	+
Saponins	-	-	-	+	-	-
Caumarine	+	+	+	+	+	+

List of Abbreviations: TSW*: Teucrium stocksianum* water extract, TSM: *Teucrium stocksianum* methanolic extract; CBW: *Chenopodium botrys* water extract; CBM: *Chenopodium botrys* methanolic extract, MBW: *Micromeria biflora* water extract; MBM: *Micromeria biflora* methanolic extract

Teucrium stocksianum) were tested for antinociceptive, antioxidant activities and analysis for chemicals composition.

The antinociceptive activity of extracts is usually evaluated in acetic acid induced writhing test (Khan *et al*., 2011b, Chavan *et al*., 2012). The acetic acid injection creating pain induction by liberating endogenous chemicals as well as some other pain mediators substance's such as arachidonic acid *via* cyclooxygenase, and prostaglandin biosynthesis (Farouk *et al*., 2008). Writhing is distinct as narrowing of the abdominal muscles attended by an extension of the forelimbs and elongation of the body (Muhammad *et al*., 2013).

The results of our study on the selected medicinal plants demonstrate significant pain reversal effects in animal model caused by injection of acetic acid. The methanolic extract of *C. botrys* showed most prominent antinociceptive effect followed by aqueous extract in a dose dependent manner therefore it can be conclude that the pharmacologically active components of *C. botrys* primarily concentrate in methanolic and aqueous extracts. The actions of extracts of *C. botrys* and *M. biflora* were more pronounced in a dose dependent manner. The presence of flavonoids and caumarines could be attributed to the blockade of the effects or the release of endogenous noxious stimulating agents at some stage and thus prevent painful sensation.

The antinociceptive action of aqueous extract of *M. biflora* was more pronounced followed by methanolic extract in a dose dependent manner. The phytochemical tests showed the presence of flavonoids, caumarines and tannins. The current finding can be due to the presence of these secondary metabolites.

DPPH is a popular spectrophotometric method for evaluation of antioxidant potential of test compounds in which the stable free radical, DPPH changes color from violet to yellow by the reduction of chemical consitutants (Khan *et al*., 2012a, Khan *et al*., 2012c). We observed scavenging effects of all three tested plants when tested on various concentrations. The crude extracts of the plants have prominent anti-radical formation potential which is strongly supported the antinociceptive activity.

In conclusion, among the selected plants (*C. botrys* and *M. biflora*) provoked significant antinociceptive effects in animal model of pain assessment, which was strongly augmented by their antioxidant effects *in-vitro*. The results validated the folk uses of these plants in management of painful conditions.

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