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EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY OF *GLYCYRRHIZA GLABRA* AND *TINOSPORA CORDIFOLIA*

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Abstract: The present study was carried out to evaluate the *in-vitro* anti-inflammatory properties of different extracts of *Tinospora cordifolia* stem and *Glycyrrhiza glabra* root in combinations by bovine serum albumin denaturation method. Chloroform and methanol extracts of both plants were prepared by maceration extraction. The anti-inflammatory activity of different concentrations of different extracts of both nutraceutical plants in combinations 2:1 and 1:2 were evaluated. Aspirin was used as standard drug. The percentage inhibition of combination of *G. glabra* and *T. cordifolia* chloroform extract in ratios of 2:1 and 1: 2 were found significant ($p < 0.05$) inhibition up to 79.45 ± 0.337 and 64.36 ± 0.27 at 25 µg/ml when compared to the control, respectively. Whereas the percentage inhibition values of methanolic extract at both plants in ratio of 1:2 and 2:1 were found significant ($p < 0.05$ inhibition up to 83.21 ± 0.024 and 71.28 ± 0.51 at 25 µg/ml, respectively. The result clearly indicates that the both nutraceutical plants in combinations 2:1 and 1:2 and methanolic extracts possess better anti-inflammatory properties.

Keywords: in vitro, inflammation, guduchi, combination, herbal formulation.

Introduction

The use of medicinal plants as a source of new drug for pharmaceutical industry has been focused since last decades. Various traditional medicinal plants are nowadays focused for development of new alternatives for allopathic drugs. According to World Health Organization, about more than 80% of the world's population including developed countries still rely on use of medicinal plants for their primary healthcare. The pharmacological action is due to various secondary metabolites present in the plant [1].

Inflammation is associated with denaturation of protein which is associated to Type III hyper-sensitive reaction. Research showed that NSAIDs are not only inhibiting the COX but also prevents the protein denaturation but it also has acute to chronic side effects. Medicinal plants are devoid of the side-effects [2].

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T. cordifolia (Miers.) is distributed throughout tropical Indian subcontinent and China. It is a widely used shrub in folk and Ayurveda belonging to the family menispermaceae. It is evaluated that *T. cordifolia* have anti-spasmodic, anti-inflammatory, anti-allergic, anti-diabetic, antioxidant properties [3]. *G. glabra* is another medicinal plant which has been used by human beings for at least 4000 years. Root has demulcent, antacid, anti-ulcer, anti-inflammatory, expectorant, tonic, diuretic, laxative and sedative properties [4].

Therefore, the screening and development of drugs for their anti-inflammatory activity is the need of the hour and there are many efforts for finding anti-inflammatory drugs from indigenous medicinal plants. The objective of the present study was to evaluate the *in-vitro* anti-inflammatory properties of different extracts of *T. cordifolia* stem and of *G. glabra* root in various proportions by bovine serum albumin denaturation method.

Material and method

Collection of plant material: *T. cordifolia* stem powder and *G. glabra* root were purchased from reliable local suppliers.

Preparation of extract: Twenty five gram of each powder was soaked in 250 mL of chloroform, methanol and water for 24 hours. After 24 hours the content was filtered with Whatman filter paper no.1 and solvents were evaporated under *vacuuo*. The extracts thus obtained were stored at 4ºC until use.

Qualitative phytochemical screening was performed to check the presence of various phytochemicals like alkaloid, glycoside, saponin, flavonoid, tannins *etc*. [5].

1mg/mL solution was prepared by dissolving 30 mg extract in 30 mL Milli-Q water. Chloroform extract was dissolved by incorporating 5 to 10% DMSO as a solvent. Suitable dilutions were made from this stock solution with Milli-Q water only. Each extract solution was mixed in the proportion of 1:2 and 2:1 with respective extracts.

Anti-inflammatory activity: An anti-inflammatory activity was determined using by inhibition of albumin denaturation method [6, 7]. 1 mL of sample solution of different concentrations (10, 15, 20 and 25 µg/ml) was added to 1mL (0.1%) of bovine albumin fraction and 1 mL TRIS-HCl buffer pH 7.8 solution. This solution was incubated for 20 minutes and allowed to heat at 72^0C for 2-4 minutes in water bath than cooled at room temperature. Control solution contained 1 mL distilled water with 1mL (0.1%) bovine albumin fraction and 1 mL buffer solution. Aspirin was used as standard anti-inflammatory drug. The absorbance values were measured at 660 nm and converted into the percentage inhibition using the following equation:

Statistical analysis: All the data were expressed in mean ±SE (n=3). Selected data were analyzed by one-way ANOVA followed by Duncan Multiple Range Test (DMRT) to compare difference in means.

Results and discussion

In study antioxidant activities of *T. cordifolia* stem powder and *G. glabra* root powder alone with combination of both extract of methanol and chloroform were investigated by in inhibition of albumin denaturation method. Result showed that, the percentage inhibition of combination of *G. glabra* and *T. cordifolia* chloroform extract in ratios of 2:1 and 1: 2 were found significant ($p < 0.05$) inhibition up to 79.45 ± 0.337 and 64.36 ± 0.27 at 25 µg/ml when compared to the control, respectively (Table 1).

Table 1 Percent inhibition (mean ±SE) of various proportions of different extract from *G. glabra* and *T. cordifolia* by inhibition of albumin denaturation method

Name of extract or combination ratio	Concentrations (µg/mL)			
	10	**15**	**20**	**25**
Aspirin	46.83 ± 0.441^{a}	64.78 ± 0.338^{a}	68.28 ± 0.338^{b}	72.15 ± 0.21^{b}
Chloroform extract (2:1)	68.54 ± 0.144^{c}	71.47 ± 0.344^{b}	73.52 ± 0.593^{b}	79.45 ± 0.337^{d}
Methanol extract (2:1)	64.88 ± 0.52^{b}	66.54 ± 0.63^{a}	68.54 ± 0.56^{b}	71.28 ± 0.51^{b}
Chloroform extract (1:2)	35.52 ± 0.47^{a}	51.27 ± 0.20^{a}	64.06 ± 0.31^{a}	64.36 ± 0.27^{b}
Methanol extract (1:2)	75.26 ± 0.270^{c}	81.85 ± 0.536^{c}	83.15 ± 0.086^{c}	83.21 ± 0.024^{d}

Values with the same superscription in a column are not significantly different whereas, values with different superscript in a column are significantly different at p-value<0.05.

Whereas percentage inhibition values of methanolic extract at both plant in ratio of 1:2 and 2:1 were found significant ($p < 0.05$) inhibition up to 83.21 ± 0.024 and 71.28 ± 0.51 at 25 µg/ml, respectively. This may be due to the synergistic effect of phytochemicals present in both the plants i.e. flavonoids and glycosides of *T. cordifolia* and triterpenoid acid in *G. glabra.* The result clearly indicates that both nutraceutical plants in combinations 2:1 and 1:2 and methanolic extracts possess better anti-inflammatory properties. Present research might be a base for development of new herbal formulation having anti-inflammatory action.

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