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VALIDATION OF DIFFERENT METHODS COMPARING WITH THEIR SPECIFICITY

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ABSTRACT

In literature it is mentioned that different organic solvents (methanol, ethanol, water, acetone and 80% ethanol) are used for the extraction of phytochemical from plants. In this paper we attempted to analyze all these organic solvents and tried to find out that which one is the most suitable solvent for the extraction of phytochemical. One variety of chickpea genotype (JAKI9218) was collected from College of Agriculture, Sehore, Madhya Pradesh. Seed coat were removed and crushed into fine powder. Phytochemicals were isolated using soxhlet apparatus with various solvents. Extract was investigated to find active constituents of the seed extracts by the different phytochemical tests and their anti oxidant activity.

Keywords- Solvent, Phytochemicals, Chickpea.

I. INTRODUCTION

Pulses are a goldmine of novel chemicals known as secondary metabolites which are used as potent drugs in modern day [1]. In this regard, researchers have focused their much attention on pulses and their therapeutic uses. Chickpea (*Cicer arietinum* L.) is an important pulse commonly known as poor's man meat for protein supply [2]. In spite of its protein content it also contains phytochemicals which are regarded as potent anti oxidant and their therapeutic uses are mentioned in literature [3]. Such studies have been resulted in increased interests in research and possibly greater use of pulses in the daily diet and in a variety of food products. Isolation of phytochemicals using different solvents are mentioned in literature. In this study we attempted to analyze the different solvents and their marked effect on isolation.

II. MATERIALS AND METHODS

Materials- The seeds of chickpea (*Cicer arietinum* L.) were obtained from College of Agriculture, Sehore, Madhya Pradesh Indore.

Methods-Total phenolic content (TPC) of each sample was estimated using the Folin–Ciocalteu colorimetric method using Mallick and Singh [4]. Phytic acid was estimated following [5]. Tannins were measured as tannic acid equivalents [6]. For antioxidants activity procedures described by Tomoyuki [7] and Chidambara et al., [8]. Various antioxidant parameters including scavenging of free-radicals DPPH, hydroxyl radicle; hydrogen peroxide scavenging, total antioxidant capacity; anti-lipid per-oxidation and anti-hemolytic activity was done according to the standard procedure of [9].

Statistical Analysis

All work was done in triplicates and the data presented are means \pm S.D. of three independent determinations. Significance was accepted at $p > 0.05$.

III. RESULTS AND DISCUSSION

Phytochemical analysis of using different solvents show considerable amount of tannins, phenolics and phytic acid. The highest concentration of tannin was found in water extract (1.78 ± 0.03) followed by 80% ethanol extract (1.08 ± 0.04) and least concentration were observed in acetone (0.29 ± 0.03). The trend obtained in case of tannin is as

follows water > 80% ethanol > methanol > acetone (Table.1). The phytic acid concentration varies widely in studied sample. The highest concentration was found in methanolic extract (0.85±0.04) followed by 80% ethanol (0.69±0.02) and least amount was reported in acetone (0.08±0.03). The total phenolics contents also vary indifferent solvents. The highest concentration was found in methanolic extract (1.78± 0.03) followed by water extract (1.89± 0.05) and least amount was reported in acetone (0.19± 0.04) (Table.1). The antioxidant activities of methanolic extract showed the highest scavenging activity followed by 80% ethanol and least activity is shown by acetone extract.

Table. 1 Phytochemicals of chickpea and their Antioxidant activities.

Cultivars	Solvents	Phytic acid (mg/gm)	Phenolics (mg/gm)	Tannin (mg/gm)	DPPH (% Inhibition)	H ₂ O ₂ (% Inhibition)	Total AO (% Inhibition)	Egg Albumin (% Inhibition)	Anti-hemolytic activity (% Inhibition)
JAKI9218	Methanol	0.85±0.04	1.78±0.03	1.49±0.05	58.39	28.76	39.55	29.78	87.56
	Ethanol	0.56±0.04	0.66±0.04	0.45±0.03	19.39	13.65	22.53	1.79	23.96
	80% Ethanol	0.69±0.02	1.55±0.03	1.08±0.04	45.78	19.67	33.57	22.68	73.74
	Water	0.42±0.04	1.89±0.05	1.78±0.03	56.42	57.49	40.33	27.47	56.76
	Acetone	0.08±0.03	0.19±0.04	0.29±0.03	6.75	3.88	2.69	10.29	8.66

(Significant at (p ≤ 0.05), All values are the average of three determinations Means± standard deviation (SD).

IV. CONCLUSION

Among all the solvents tested for plant phytochemicals methanol showed the highest activity of anti oxidants property but we find an interesting facts that water is the ultimate solvents for plant phenolics and tannins which are reported as pharmacological activity.

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