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In vitro qualitative analyses of different phytoconstituents in aqueous, ethanolic and hydro-ethanolic extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material

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Abstract
Carica papaya and Andrographis paniculata are the well-known medicinal plants in the world. The powdered dried leaves of *Carica papaya* and whole plant of *Andrographis paniculata* were subjected to extraction by columnar Soxhlet extraction apparatus and extracted by three different solvents such as aqueous, absolute ethanol and hydroethanol (1:1) with standard protocol. The highest yields were obtained from the aqueous (16% and 13.5%), followed by hydroethanolic (14.24% and 12.75%) and lowest from absolute ethanolic extracts (12% and 11.1%) of *Carica papaya* leaves and *Andrographis paniculata* plant material, respectively. Alkaloids, saponins and tannins were present in ethanolic and hydroethanolic extracts but they were absent in aqueous extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material. Phenolic compounds, cardiac glycosides and flavonoids were present in aqueous, ethanolic and hydroethanolic extracts whereas carbohydrates (reducing sugars) and steroids were absent in all these extracts of both plants. The presence of these phytoconstituents in the extracts of these plants indicate that they can be used medicinally.

Keywords: Alkaloids, flavonoids, saponins, tannins, phenolic compound, cardiac glycosides

Introduction
Carica papaya (Family Caricarceae) is a well-known plant in the world [32]. It is a soft wooded single-stemmed perennial tree, 2-10 m in height, with a crown of large palmate from the apex of the trunk [24]. Almost all parts of the papaya plants are used as therapeutic regimen in Ethno-Veterinary Medicine. For example, seeds are used as dewormers, leaves (especially fallen) for treating fever or pyrexia, wounds, diabetes and gonorrhea [18]. Extract from the leaves has the property to prevent and reverse sickle cell anaemia [21]. Crude extract of *Carica papaya* leaves enhances the thrombocyte and erythrocyte counts [13]. CPLE increases the platelet count without any side effects and prevents complications of thrombocytopenia [16]. It boosts thrombopoiesis and erythropoiesis in humans and animals [13] and also increases platelet count in chemotherapy-induced thrombocytopenia [39]. CPLE acts as hepatoprotectant and platelet count enhancer in febrile conditions [37] and also a potent health booster [38].
The main phytoconstituents of papaya leaves are carpaine (alkaloid) and carposide (glycoside) [34]. *Carica papaya* leaves contain secondary metabolites like flavonoids, alkaloids, saponins, tannins, β-carotene, glycosides and steroids having immunomodulatory, antitumor and antipathogenic activities [6]. Papaya leaves also having bioactive compounds such as chymopapain and papain that help in the process of digestion and also inhibit the growth of pathogenic microorganisms [42, 44]. Papaya leaves are rich in protease and amylase. These enzymes have high anti-inflammatory properties which reduce the inflammation of stomach and colon [5]. Papaya leaves extract heals peptic ulcers by killing bacteria *Helicobacter pylori* owing to their antimicrobial properties. Papaya leaves contain carpain, acetogenin and phenolic compounds. Carpain is a chemical compound or a substance with ability to kill microorganisms that often intervene in food digestion processes [6, 9] and also stops the excess growth of skin flora by sanitizing skin from the toxins and provides protection against skin problems like pimples, freckles and acne [41]. Phytochemical analyses have indicated that tannins, saponins, flavonoid, phenol, steroid, alkaloid, cardenolide, reducing sugar, cardiac glycoside and antraquinone are present in the extract of *Carica papaya* leaves [10, 7, 2].

Andrographis paniculata is commonly called as "Kalmegh" (Family: Acanthaceae) and referred to as a medicinal plant [35]. The plant has certain phytochemicals such as flavanoid, lactone, diterpene glycoside, diterpenoid but major phytochemicals are neoandrographolide, andrographolide, 14 deoxy 11, 12 di-deandrgrapholide, iso-andrographolide and minor phytochemicals are xanthone such as dihydroxydimethoxy xanthone and tri methoxy 1-hydroxy xanthine. It possesses various medicinal properties such as antimalarial, anticancer, antiinflammatory, antiulcer, anti-hyperglycemic, antiviral, antiangiogenic, hepatoprotective, antimicrobial, immunomodulatory, antiparasitic and antioxidant properties [11, 3, 30] and also possesses platelet enhancing, antihypertensive, cardiac protective properties and recommended in the therapy of inflammation of bronchi or bronchioles or nasal mucosa [22]. *Andrographis paniculata* (Kalmegh) seeds extract (APSE) contains diterpenes, flavonoids, xanthones, noriridoides etc. [36]. It has property of anti-microbial/protozoan, anti-inflammatory, anti-oxidant, immuno-stimulant, anti-diabetic, hepato-renal protective, liver enzymes modulator [31]. It has potent antioxidant, anti-inflammatory, hepatoprotective, anti-hyperglycemic effects on cardiovascular disease and platelet activation [23]. Keeping these points in mind, the present study was conducted to evaluate the different qualitative phytoconstituents in aqueous, ethanolic and hydroethanolic extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material.

Materials and Methods

The plant materials were collected from Bareilly province of Uttar Pradesh, India. The collected plant materials were identified by the Department of plant science, Rohilkhand University, Bareilly. The collected plant materials were washed three times using distilled water to clean and remove the waste materials and shade dried at normal room temperature for 15-20 days. The dried plant materials were pulverized into fine powder and stored in air tight containers to avoid contact with moisture. The powdered plant materials were subjected to extraction by columnar Soxhlet extraction apparatus and extracted by three different solvents such as aqueous, absolute ethanol and hydro-ethanol (1:1) with standard protocol. The dried extracts of different types of solvents were weighed, yields were calculated and preserved at -20°C for further use. The presence of alkaloids was determined by the Mayer's test, Hager's test and Wagner's test [15]; the phenolic compounds by the lead acetate test and ferric chloride test [28]; the proteins by the biuret test (Gahan, 1984) and ninhydrin test [43]; carbohydrate and glycosides by Fehling's test [33], Benedict's test [33] and Keller-Kiliani test [26]; saponins by foam test [12]; flavonoids by alkaline reagent test [19], steroids by Salkowski Test [19] and tannins by lead acetate test [26] and Braymer's Test [4].

Results

Table 1: Extraction yields by the Soxhlet apparatus

S. No.	Solvent	Extract Yield (%)	
		C. papaya leaves	*A. paniculata* plant material
1.	Aqueous	16.0	13.5
2.	Absolute Ethanol	12	11.1
3.	Hydroethanol (1:1)	14.24	12.75

Table 2: *In vitro* qualitative phytochemical analyses of plants extracts

Phytochemical	*C. papaya* leaves			*A. paniculata* plant material		
	Aqueous	Ethanolic	Hydro-ethanolic	Aqueous	Ethanolic	Hydro-ethanolic
1. Alkaloids						
• Mayer's test	-	+	+	-	-	-
• Wagner's test	-	+	+	-	+	+
• Hager's test	-	+	+	-	+	+
2. Phenolic compounds						
• Lead acetate test	+	+	+	+	+	+
• Ferric chloride test	+	+	+	+	+	+
3. Proteins	-	-	-	-	-	-
4. Carbohydrates & glycosides						
• Fehling test	-	-	-	-	-	-
• Benedict test	-	-	-	-	-	-
• Libermann's test	-	-	-	-	-	-
• Molisch's test	-	-	-	-	-	-
• Keller-Killani test	+	+	+	+	+	+
5. Saponins						
• Foam test	-	+	+	-	+	+
6. Flavonoids						
• Alkaline reagent test	+	+	+	+	+	+
7. Steroids	-	-	-	-	-	-
8. Tannins						
• Braymer's test	-	+	+	-	+	+

(+) Present, (-) Absent

The yields from different types of extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material were shown in Table 1. The highest yield was obtained from aqueous extract (16% and 13.5%), followed by hydroethanolic extract (14.24% and 12.75%) and lowest from absolute ethanolic extract (12% and 11.1%) of *Carica papaya* leaves and *Andrographis paniculata* plant material, respectively. *In vitro* phytochemical analyses of different types of extracts were performed as per standard procedures and the results were shown in Table 2. Alkaloids, saponins

and tannins were present in ethanolic and hydroethanolic extracts but they were absent in aqueous extract of *Carica papaya* leaves. Phenolic compounds, flavonoids and cardiac glycosides were present in aqueous, ethanolic and hydroethanolic extracts of *Carica papaya* leaves. Proteins, carbohydrates and steroids were absent in aqueous, ethanolic and hydroethanolic extracts of *Carica papaya* leaves.

Discussion

In the present study, the highest yield of *C. papaya* leaves and *A. paniculata* plant material extracts were found in aqueous solvent followed by in hydro-ethanol solvent and lowest in absolute ethanol. There are numerous procedures, including milling, grinding, extraction and homogenization, to acquire phytoconstituents from the plant [14]. The crucial stage in these processes for recovering and isolating phytoconstituents from the plant material is extraction. The method, time, temperature, composition of phytoconstituents and solvent employed all have a significant impact on the extraction's effectiveness but the type of solvent plays an important role in the extraction yields [29]. The findings demonstrated that different solvents had different extraction yields. The variations in the polarity of the extraction solvents result in a wide range in the concentration of phytoconstituents in the extract. It may be due to the high concentration of polar phytoconstituents in the plants, which are soluble in highly polar solvents including water, methanol, and ethanol [27].

In our study, phenolic compounds, cardiac glycosides and flavonoids were present in aqueous, ethanolic and hydro-ethanolic extracts while alkaloids, saponins and tannins were present only in ethanolic and hydroethanolic extracts of *C. papaya* leaves and *Andrographis paniculata* plant material. The qualitative analyses of different phytoconstituents provide an important clue about the kind of phytoconstituents present in the plant [25]. The phytoconstituents such as polyphenolics and flavonoids are more efficiently extracted in polar solvents including water and ethanol [40]. The alkaloids, phenolics, terpenoids and flavonoids are highly soluble in alcoholic solvent than the other [14, 8] reported the presence of alkaloids, carbohydrates, tannins, flavonoids, steroids and phenolic compounds in methanolic extracts and absence of alkaloids, steroids and flavonoids in aqueous extracts. [20] reported the presence of alkaloids, terpenoids, carbohydrates, tannins, flavonoids and steroids in chloroform and alcohol extracts of *Carica papaya* leaves and absence of alkaloids, flavonoids and steroids in aqueous extract. The saponin was more efficiently extracted in water-alcohol mixture than pure water or pure alcohol [1].

Conclusion

It was concluded that the ethanolic and hydro-ethanolic extracts of *Carica papaya* leaves and *Andrographis paniculata* plant material contain more phytoconstituents as compared to aqueous extract but the yields were slightly less as compared to aqueous extracts.

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