

Bioassay Guided Screening of *Cryptolepis* Extracts for Onward Synthesis of Nano Ferrites

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Article Info

Article history:

Received 10 October 2021

Received in revised form 03
December 2021

Accepted 12 December 2021

Keywords:

Cryptolepis Buchanani

Phytochemistry

Plant Analysis

Phytochemicals

Nano Ferrites

Nickel-Zinc Compounds

Abstract

In this study, various leaves and stem organic extracts were prepared using the solvents viz., *n*-hexane, ethyl acetate, ethanol and water, separately. Different phytochemical test for alkaloids, carbohydrates, oils, amino acids and others were performed to determine their presence in the extracts. These plant extracts were used for screening of sample via UV and HPLC techniques to compare the wavelength and absorbance, and retention time on chromatogram by extracts, respectively. The surface properties and size of nickel-zinc nano ferrites were evaluated by Energy-dispersive X-ray spectroscopy (EDX) and Scanning Electron Microscopy (SEM). The former, showed the presence of nickel (Ni) and zinc (Zn) weighing 4.27% of N, 6.89% of C and 35.5% of O in the sample which confirmed the presence of nano ferrites in leaves and stem of *C. buchanani*.

Introduction

The *Cryptolepis buchanani*, commonly known as Kareballi (Dahotre & Singh, 2011), is being cultivated in palaeotropic and its Components like sarverogenin and isosarverogenin (Kumar et al., 2014; Ghosh et al., 2008; Hanprasertpong et al., 2017). have powerful cytotoxic action and are anti-inflammatory as well as show immunopotent characteristics (Maravajhala et al., 2012). Major components of *C. buchanani*'s leaves are alkaloids (Jang et al., 2013), amyirin, carbohydrates, saponins, steroids and triterpenes (Mehri et al., 2012; Mohanpuria et al., 2008). while stem contains alkaloids and triterpenes (Sivakumar et al., 2011; Raghavender & Jadhav, 2009).

A potential impact of *Cryptolepis buchanani* is synthesis of nanoferrites using *C. buchanani* leaves extracts with ethylacetate nanoferrites (Sharma & Pallavi, 2012). The nano ferrites can be characterized by using various techniques like scanning electron microscopy (SEM). The structural, electronic, magnetic, optical characteristics (Sharma et al., 2012) of nano ferrites are proved to be far better than their large surface and quantum confinement relative to their ferrites in bulk (Pande et al., 2006). The study was designed to analyze the presence of nano ferrites in *Cryptolepis buchanani* leaves and stem extracts through different methods.

Methods

The solvents used for experimental purpose were standard. Some solvents were purchased from Delta Lab Lahore, while others were available in chemistry lab University of Lahore, Sargodha campus.

Experimental design

At first, the plant sample of *Cryptolepis buchanani* were collected from the farm, washed to remove dust and other impurities, and separated from the whole and dried, afterwards. Then, various parts of the selected plant were ground to obtain fine samples and kept in different vials after labelling. The extracts of *Cryptolepis buchanani* leaves and stems prepared by Soxhelt apparatus using organic solvents such as n-hexane, ethanol and water. Finally, different biochemical tests were performed to check the presence of various phytoconstituents in these extracts and confirmed by SEM and EDX analysis.



Figure 1. Preparation of leaf extract of *Cryptolepis buchanani*

Cryptolepis buchanani extracts were labelled as

- *Cryptolepis buchanani* leaves extracts with n-hexane (CBEL-H)
- *Cryptolepis buchanani* leaves extracts with ethylacetate (CBEL-Ea)
- *Cryptolepis buchanani* leaves extracts with ethanol (CBEL-E)
- *Cryptolepis buchanani* leaves extracts with water (CBEL-W)
- *Cryptolepis buchanani* stem extracts with n-hexane (CBEL-H)
- *Cryptolepis buchanani* stem extract with ethylacetate (CBEL-Ea)
- *Cryptolepis buchanani* stem extract with ethanol (CEBL-E)
- *Cryptolepis buchanani* stem extract with water (CBEL-W)

Synthesis of Nix Zn_{1-x} Fe₂O₄ nanoferrites

In this study, Nix Zn_{1-x} Fe₂O₄ altered sol-gel technique was used to synthesize nanoferrites. *Cryptolepis buchanani* plant extract was typically combined with distilled water under strong agitation to ensure the production of homogenous solution, while maintaining pH. Then, heating on the hot plate at 100°C under strong mixing, mixture was allowed to evaporate for a couple of hours until a dehydrated originator was attained. This dehydrated mass was crumpled into powder by mortar and pestle

Results and Discussion

Phytochemistry of *Cryptolepis buchanani* extracts

All extracts of *Cryptolepis buchanani* were subjected for further qualitative analysis to detect the presence of various compounds. Different phytochemical tests were performed, and results obtained from experiments.

Table 1. Phytochemical analysis tests of *Cryptolepis buchanani* leaves and stem extracts

“+” sign is for illustration of functional groups presence.

“-” sign is for illustration of functional groups absence.

Phytochemical Detection	Tests	Measurments (Leaves)				Measurements (Stem)			
		CBELH	CBELEa	CBELE	CBE L-W	CBESH	CBESEa	CBESE	CBESW
Test For Alkaloids	Mayer's Test	++	-	++	++	++	-	++	-
	Wagner's Test	++	++	++	++	++	++	++	++
Amino Acids	Ninhydrin test	-	-	++	++	-	-	++	++
Carbohydrates	Molish's test	++	++	++	++	++	++	++	++
	Benedict's test	++	++	++	++	++	++	+	++
Oils and Fat	Spot test	-	++	++	++	-	++	++	-
	Saponification	-	++	++	+	-	++	++	-
Phenolic Compounds	FeCl ₃ Test	++	-	++	++		-	++	++
	Lead acetate test	++	-	+	-	++	-	++	-
	Alkaline reagents	++	++	-	++	++	++	-	++
Glycosides	Borntrager's test	++	-	-	-	++	++	-	-
Protiens	Biuret test	++	++	++	++	++	++	++	++
Saponins	Foam test	-	-	++	++	-	-	++	++
Volatile oil	Oil test	++	++	++	-	++	++	++	-

UV spectroscopic analysis

The UV visible spectroscopy has examined the catalytic activities of samples. Extracts of *C.buchanani* was subjected to UV analysis and maximum absorbance for each sample was observed. It was evident that extracts of *C.buchanani* from ethyl acetate extracts were the most active in all samples and *CBEL* showed peak absorption and wavelength.

Table 2. Comparision of UV analysis between *C.Buchanani* extracts.

Samples	Wavelength(nm)	Absorbance
<i>C.Buchanani</i> leaves extract with ethylacetate	576.80	0.234
	486.40	2.266
	312.00	2.035
<i>C.Buchanani</i> leaves extract with ethanol	483.00	0.366
	398.60	0.273
	443.20	0.045
<i>C.Buchanani</i> stem extract with ethanol	417.80	0.264
	410.60	0.284
<i>C.Buchanani</i> leave with water	364.20	0.480

Estimation of chemical compounds of *Cryptolepis buchanani* leaves extracts by using HPLC

HPLC chromatogram ethanolic leaves extract of *Cryptolepis buchanani* with ethylacetate showed a variety of peaks showing that nine compounds were present. The chemical compounds found in ethanolic extract of *Cryptolepis buchanani*.

Table 3. HPLC analysis of *Cryptolepis Buchananani* leaves extract in ethylacetate

Peak no.	Retention time	Name of compounds	Molecular Formula
1	7.870		
2	8.320		
3	9.132	D-Galactose,6deoxy-[DFucose]	C ₆ H ₁₂ O ₅
4	9.434		
5	10.942	Dodecanoic Acid	C ₁₂ H ₂₄ O ₂
6	11.132	Phosphonofluoric acid, (1methyl ethyl) cyclohexyl ester	C ₉ H ₁₈ FO ₂ P
7	14.602		
8	16.822	n-Hexadecanoic Acid	C ₁₆ H ₃₂ O ₂
9	17.289	Unknown	
10	18.742	9,12octadecadienoic acid	C ₁₈ H ₃₂ O ₂
11	18.856	Oleic acid	C ₁₈ H ₃₄ O ₂
12	19.255	Octadecanoic acid	
13	19.398		
14	21.023	4,8,12,16tetramethyl heptadecan-4olide	C ₂₁ H ₄₀ O ₂
15	22.068		

Fourier Transform Infrared (FT-IR) analysis of *Cryptolepis buchanani*

FT-IR analysis has been performed for *Cryptolepis buchanani* extracts and was used to classify diverse functional groups and compounds present.

Table 4. Comparison for FT-IR analysis of *C.buchanani* extracts

“+” sign is for illustration of functional groups presence.

“-” sign is for illustration of functional groups absence.

Sr. NO	Functional Groups	CBEL-H	CBEL-Ea	CBEL-E
1	Alkane	++	++	++
2	Alkene	++	-	++
3	Alkyne	++	-	-
4	Ketone	++	++	-
5	Ester	++	++	-
6	Alcohol	++	-	++
7	Phenol	++	-	-
8	Ethers	++	-	-
9	Cyanide	-	-	-
10	Amide	-	++	
11	Azo	++	++	++

12	Amine	-	++	++
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Nickel zinc nano ferrites detection

Nickel zinc nano ferrites based on the extract of *Cryptolepis* (SEM) were viewed in scanning electron microscopy for analysis of surface morphology. The forms of nan ferrites are evident as the SEM images show that the particles are spherical in form of Nickel-zinc. Similarly, the EDAX method was used to analyse nickel-zinc nano ferrites and the chromatogram showed that nickel (Ni) and zinc (Zn) were presents in pure form.

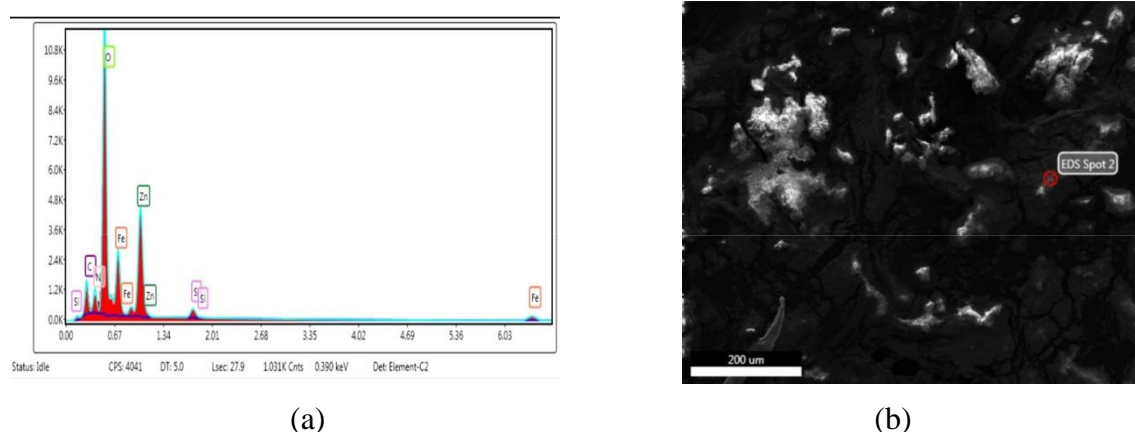


Figure 1. (a) EDAX. (b) SEM view

Conclusion

The synthesis of nickel-zinc nano ferrites with *Csryptolepis buchanani* extracts would be free of toxic contaminants and would open the way for cheaper chemicals. The plant extract-based synthesis can provide nano ferrites of a controlled size and morphology. In conclusion, nickel-zinc nano ferrites synthesized from the *Cryptolepis buchanani* leaf extracts confirmed that leave extract prepared using ethyl acetate was the most active of all solutions.

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