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UV / HPLC-MS identification of phenolic constituents from *Ageratum conyzoides* L.

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Abstract

This work presents the Spectro-analytical studies of phenolic constituents from *Ageratum conyzoides* L. plant. The Phenolic Compounds play an important role in the plants. They serve as defences against different herbivores and microbes. As all the phenolic compounds such as flavonoids, legnines, tannins etc contains at least one aromatic ring with hydroxyl moiety. Keeping in view its importance and structure of the phenolic compounds the present study was carried out to isolate such compounds. Firstly the plant extracts were prepared by standard extraction methods. Then, the separation of the phenolic constituents was done by using simple chromatographic techniques such as fla sh column chromatography (FCC) and preparative thin layer chromatography (PTLC) from each plant extract. The type of sugars present and the presence or absence of flavonoids were confirmed by acid hydrolysis and flavonoid tests. The identification, differentiation and structure elucidation of the pure flavonoids and phenolic acids were achieved by High Performance Liquid Chromatography technique coupled with Mass Spectrometry (HPLC-MS). The results showed that the extracts contain a phenolic acid and flavonoid derivatives. The results clearly revealed that in future one can use the simple chromatographic techniques for the determination of Phenolic compounds from plants.

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Introduction

The *Ageratum conyzoides* L. (Asteraceae) consists of approximately 30 species, but only a few species have been phytochemically investigated.

The genus contains a wide range of bioactive chemical constituents, including alkaloids (Wiedenfeld *et al.,* 1991), cumarins (Desai *et al.*, 1973), flavonoids (Adesogan *et al.,* 1979), chromenes (Pham *et al.,* 1976), benzofurans (Pari *et al.,* 1998), sterols (Dubey *et al.,* 1989) and terpenoids (Horng *et al.,* 1976).

Various plant extracts of these plants have been found to possess pharmacological and insecticidal activities (Anooj *et al.,* 2008). A. conyzoides is traditionally used in folk medicine in various parts of the world, including Africa, Asia and South America (Singh *et al.,* 2018).

The essential oils extracted from this plant are currently used as an insecticide, especially against Acari, stored product insects and mosquito (Zoubiri *et al.,* 2014).

The plant extract is found to have cardiovascular depressant, antispasmodic, antioxidant and insecticidal activities (Nour *et al.,* 2010).

The aqueous extract of the leaves of the plant significantly reduce the formation of gastric lesion (Awaad *et al.*, 2013).

Taking into consideration the immense importance of the plant because of their diverse biological and pharmacological applications, the present work was therefore carried out to explore the phenolic constituents of *Ageratum conyzoides* by simple UV/ HPLC-MS techniques.

Methodology

The plant materials were air dried under shade, chopped and powdered. The powdered plant (5 kg) was dipped in 2 L EtOH (WR-1) for 7 days. The residue then successively subjected to extraction with 2 L EtOH (WR-2) for 14 days and with 2 L of EtOH: H_2O (WR-3) for 15 days. As a result, 40 g Ethanolic and 50 g Hydroethanolic extracts were obtained.

Each extract was subjected to the phytochemical screening and the presence of flavonoids were confirmed in each extract by different tests (Shibata Test).

Ethanolic extract 2 was subjected to acid hydrolysis in order to identify different sugars present in the plant.The hydrolyzed solution was extracted with ethyl acetate. Aqueous fraction was analyzed for sugars identification by taking comparative TLC with different sugars using silica gel as stationary phase and standard solvent system (Acetone:H_2O 9:1). Ethyl acetate fraction was analyzed by HPLC-MS.

All the extracts were subjected to preparative separation and purification by the FCC. For this purpose silica gel was used as stationary phase and first elution was carried out with toluene to separate non-polar compounds and two fractions were collected and then further elution was carried out with an increasing proportion of MeOH in order to separate polar compounds and four fractions were collected.

Purity of each fraction was checked by taking collective TLC in Toluene: Pyridine: NH_3 (80:20:1). Selected fractions were further purified by PTLC.

Detail account of purification work is given in Table.1.Identification and differentiation of compounds present in different fractions (collected by FCC) and sub fractions (collected by PTLC) were done with the help of UV spectrophotometer and HPLC-MS.

Results and discussion

Results of acid hydrolysis of ethanolic extract (WR-2) indicated the presence of glucose, xylose and rhamnose sugars in the plant extracts.

Phytochemical screening also proved the presence of phenolic acids and flavonoids in all three extracts.

Table 1. Preparative separation and purification of all three extracts.

Extract	Elution Gradiant	FCC fraction	Subfractions by PTLC	Compounds identified by UV and HPLC-MS
WR-1	100% Tol.	WR1-FF1	-	
	100% Tol.	WR1-FF2	-	
	20% MeOH	WR1-FF3	WR1-FFF3 Tol.:MeOH (9:3)	Gallic acid derivative
	40% MeOH	WR1-FF4	-	
	60% MeOH	WR1-FF5	WR1-FFF5 Tol.:Pyridine:NH_3 (80:20:1)	5,7,2'-Trihrdroxy flavone derivative
	80% MeOH	WR1-FF6	-	
WR-2	100% Tol.	WR2-FF1	-	
	100% Tol.	WR2-FF2	-	
	20% MeOH	WR2-FF3	WR2-FFF3 Tol.:MeOH (9:3)	Gallic acid derivative
	40% MeOH	WR2-FF4	-	
	60% MeOH	WR2-FF5	-	
	80% MeOH	WR2-FF6	WR2-FFF6 Tol.:Pyridine:NH_3 (80:20:1)	5,7,2'-Trihrdroxy flavone derivative
WR-3	100% Tol.	WR3-FF1	-	
	100% Tol.	WR3-FF2	-	
	20% MeOH	WR3-FF3	WR3-FFF3 Tol.:MeOH (9:3)	Gallic acid derivative
	40% MeOH	WR3-FF4	-	
	60% MeOH	WR3-FF5	-	
	80% MeOH	WR3-FF6	WR3-FFF6 Tol.:Pyridine:NH_3 (80:20:1)	5,7,2'-Trihrdroxy flavone derivative

Table 2. Mass spectrometry data.

Extract	t_R min	Molecular ion peak	Compounds	Fragments m/z	UV λ_{max} (nm)
WR-1	1.69	$[M+H_3O]^+$ 483	4-*O*-[*β*-D-glucosyl-(1→4)]-*β*-D-xylosylgallic acid	401[M+MeOH+H-$^{0,3}X_0$ xly-$2H_2O]^+$/ $[464+32+1-60-36]^+$	250
WR-2	1.76	$[M+Na]^+$ 543	4-*O*-[*β*-*D*-glucosyl-(1→4)]-*α*-*L*-rhamnosylgallic acid	381[M+Na-glc]$^+$/$[520+23-162]^+$	267
WR-3	8.17	$[M+K]^+$ 545	4-*O*-[3'-acetyl-*β*-*D*-glucosyl-(1→4)]-*β*-*D*-xylosylgallic acid	413[M+K-xyl]$^+$/$[506+39-132]^+$	262

Table 3. Mass spectrometry data.

Extract	t_R min	Molecular ion peak	Compounds	Fragments m/z	UV λ_{max} (nm)
WR-1	36.59	$[M+Na]^+$ 617	5,7,2'-Trihydro-7-*O*-[*β*-*D*-glucosyl-(1→4)]-*β*-*D*-glucoside	235[M+H-2glc-$2H_2O]^+$/ $[594+1-324-36]^+$	280, 325
WR-2	30.47	$[M+MeOH+2H]^+$ 610	5,7,2 '-Trihydro-7-*O*-[*β*-*D*-xylosyl-(1→4)]-*β*-*D*-xyloside flavone	375[M+MeOH+H-xyl-$^{0,3}X_0$ glc-$H_2O]^+$/$[576+33-132-60-18]^+$	264, 298
WR-3	37.3	$[M+Na]^+$ 719	5,7,2'-Trihydro-7-*O*-[*β*-D-xylosyl-(1→4)-*β*-*D*-xylosyl-(1→4)]-*β*-*D*-glucoside	651[M+MeOH+H-$^{0,3}X_2$ xyl-$H_2O]^+$/$[696+32+1-60-18]^+$	280, 323

Fractions obtained by FCC from each extract were subjected to collective TLC (Toluene.: Pyridine.: NH_3, 80:20:1) and fractions FF3 and FF5 in case of WR-1 and fractions FF3 and FF6 in case of both WR-2 and WR-3 was selected on the basis of the relatively simpler TLC profile.

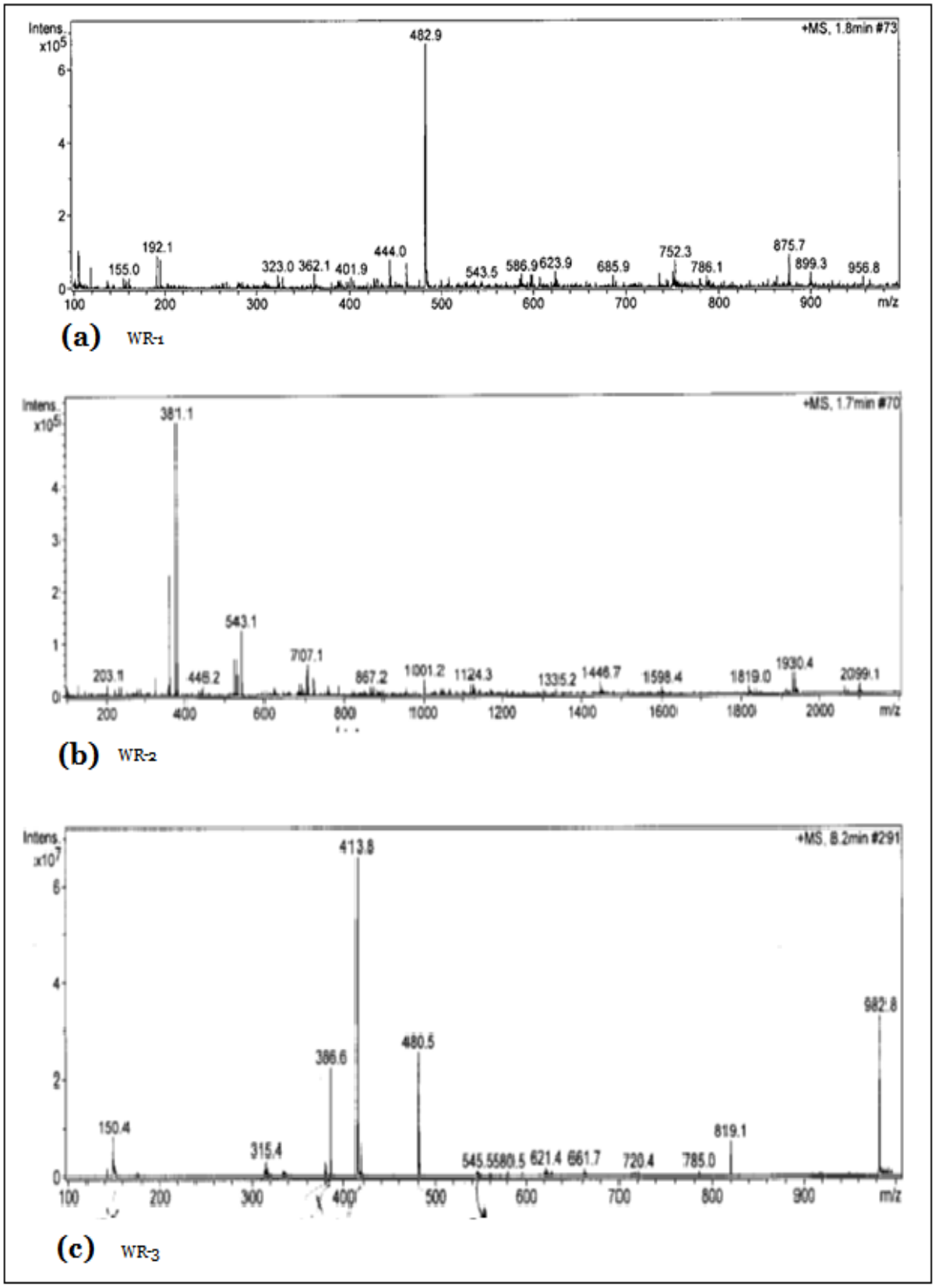

Fig. 1. ESI-MS of Gallic acid derivatives present in (a) AWR1, (b) WR-2 (c) WR-3.

Fraction FF3 in all three extracts showed the same band at same R_f value (R_f = 0.47). Fraction FF5 of WR-1 and fraction FF6 of WR-2 and WR-3 also showed one same band at same R_f value (R_f = 0.62). UV spectra of compounds purified by PTLC from the FF3 fraction of each extract showed similar single absorption band and maximum of absorption; λ_{max} = 250 nm (WR-1) ;λ_{max} = 267 nm (WR-2) ; λ_{max} = 262 nm (WR-3)Gallic acid showsλ_{max} at 270 nm (A.Romani *et al* 2012).

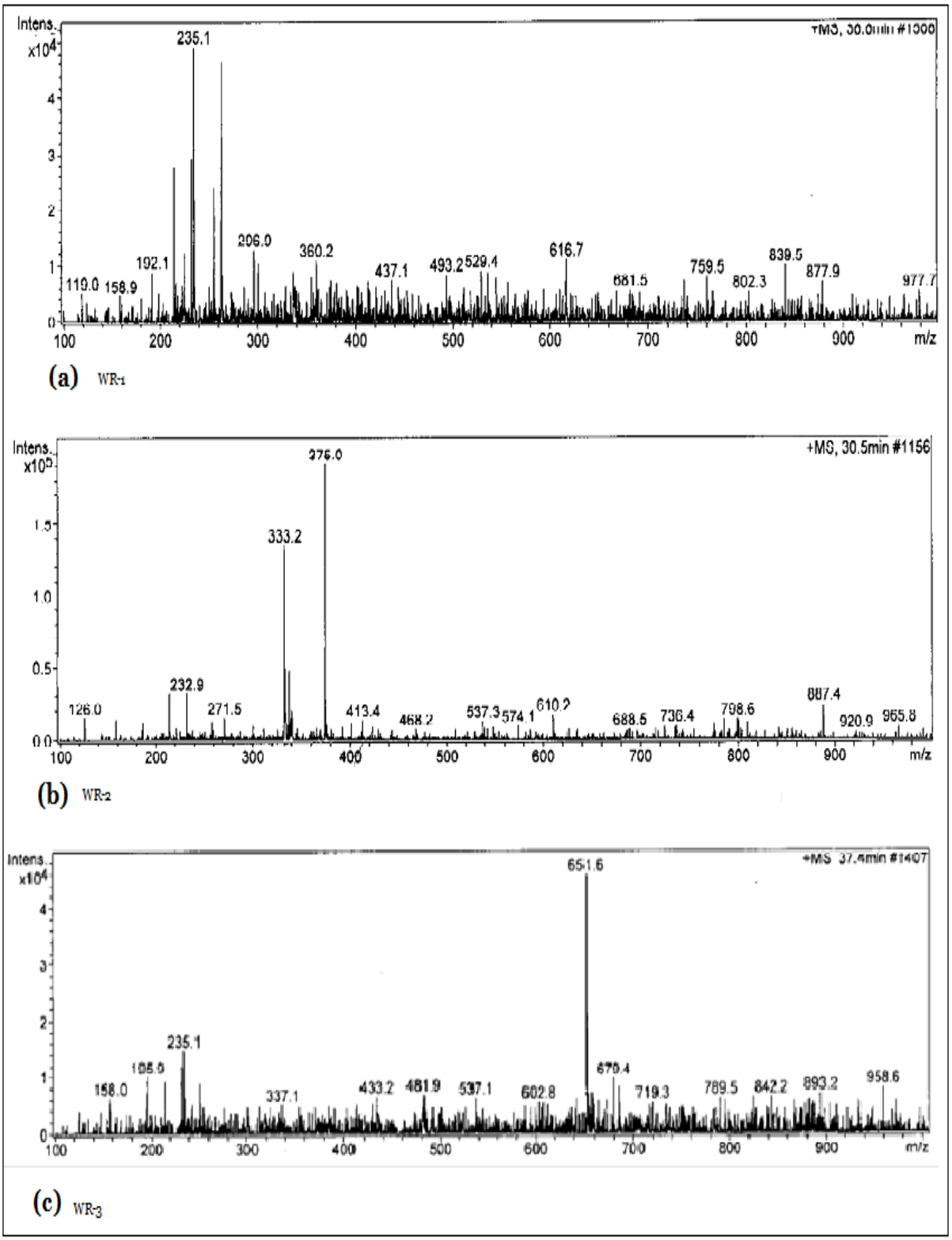

Fig. 2. ESI-MS of compound 5,7,2'-trihrdroxy flavones present in (a) WR-1, (b) WR-2 (c) WR-3.

Hypsochromic shift may be due to glycosylation of gallic acid that shifts the λ_{max}value towards lower wavelength (T.J.Mabry.*et al*,2012). From the UV data it was already established that compound purified from FF3 fraction of each extract is gallic acid derivative [Fig. 3 (a)] further confirmed by HPLC-MS analysis (Fig. 1, Table 2).

The UV spectra of compounds purified by PTLC from the FF5 fraction of WR-1 and FF6 fraction of both WR-2 and WR-3, each extract showed a similar double band pattern and maximum of absorption; λ_{max} = 280, 325 nm (WR-1), λ_{max} = 264, 298 nm (WR-2), λ_{max} = 280, 323 nm (WR-3).

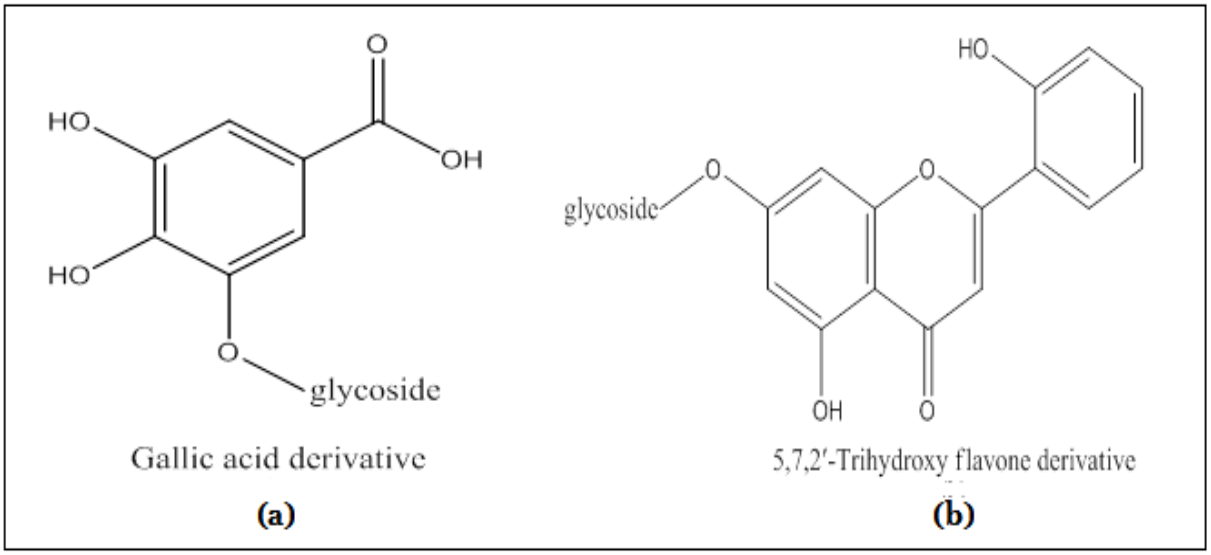

Fig. 3. (a) and (b) structure of compounds present in all three extracts.

The compound 5,7,2'-trihrdroxy flavone shows λ_{max} of band I at 266 nm and band II at 325 nm. Hypsochromic shift may be due to glycosylation of gallic acid that shifts the λ_{max} towards lower wavelength. From the UV data it was already established that compound purified from FF5 fraction of AE-1 and FF6 fraction of both AE-2 and AW is 5,7,2'-Trihrdroxy flavone [Fig. 3 (b)] derivative it was further confirmed by HPLC-MS data (Fig. 2,Table 3).

Conclusion

The experimental results showed that all the three extracts (WR-1, WR-2 and WR-3) contain a common phenolic acid **(**and flavonoid glycoside which is a 5, 7,2'-trihrdroxy flavone). As phenolic compounds are the important phytochemicals found in all types of medicinal plants. From this research it is concluded that in past most complicated methods were adopted for the isolation of the phenolic constituents, the spectroanalytical techniques is very simple and time consuming as compared to other method. Further investigation may improve the methods of isolation.

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