JNPA. XXVI(1) 2012

Research

Evaluation of the phytochemical profile and uterine contractile effect of *Woodfordia fruticosa* (L.) Kurz and *Dipsacus mitis* D. Don

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Abstract

The phytochemical screening of *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz revealed the presence of bioactive secondary metabolites in both of the species but their concentrations were variable. Flavanoids and saponins were found in remarkable amount in both of the samples. The volatile compounds of both plants were extracted by Simultaneous Steam Distillation and Extraction (SDE) apparatus and analyzed by Gas Chromatography/Mass Spectrometery (GC-MS). Total 53 volatile compounds were tentatively identified from roots of *Dipsacus mitis* D. Don and 78 were identified from flowers of *Woodfordia fruticosa* (L.) Kurz. Alcohols were dominant in both the plants accounting 45% and 32% respectively. Numerous bioactive volatile compounds were detected in both herbs. The effects of increasing concentrations of the methanolic extracts on the amplitude and frequency of spontaneously contracting uterine tissues were tested. The effect of extracts on the smooth muscle strips from rat uterus showes the dramatic muscular relaxation on spontaneous contractility and was determided most effective at a concentration 6500 μg/ml of methanolic extract of *Dipsacus mitis* D.

Key words: Nepalese herbs, secondary metaboloites, GC-MS, uterine cell contractility

Introduction

An increasing reliance on the use of medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [1]. Wide molecular diversity of plant metabolites throughout the plant kingdom represents an extremely rich biogenic resources, some of them are synergistic, some antagonistic, some toxic and some inactive [2]. Today there are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. To understand the actual value of plant remedies, several phytochemical surveys have been carried out which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids, flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils [3]. The majority of traditional medicines used in developing countries have not been evaluated for quality, safety, efficacy to same standards as those developed countries. Nevertheless, there are some remarkable claims made for their effectiveness during the practice of traditional medicines. Therefore the interest has given in scientifically validating the chemistry of plant drugs for dissolving the actual value of folkloric remedies, their efficacy and safety.

Ethnomedicinal knowledge persists, and is being transferred to the next generation in several parts of

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Nepal [4]. It is estimated that only 12-20% of the population living in around the urban area has access to the modern medicine facility and rest has to depend on the traditional medicine [5]. Crude-drugs are commonly given in the form of powder, decoctions, and infusions or in ointment forms. The herbal medicines are applied externally on cuts, wounds, boils, pimples, ringworms, muscular swelling and dislocation of bones. The single plant or plant parts such as root, rhizome, stem, leaf, bark, wood, gum, latex, ash, flower, fruit and seed, or admixture of different species of plants are recommended for treatment.

The plants *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz are reported in Nepalese literatures for their uses typically in pregnancy cases [6]. In recent past, much attention has been paid to record folk medicines of various tribal pockets and rural populations of Nepal. But the phytochemical screenings and biological activities of many of these herbs are quite poorly studied. Hence the investigation on phytochemical profiles and biological activities of Nepalese medicinal plants is in urgent need for the traditional medicinal herbs, therapeutic benefits and their possible toxic effects.

Material and Methods

Collection and identification of plant materials

Dried medicinal plants were collected from local market at Kathmandu, Nepal during 2006. All the plant samples were identified by the authors. The voucher specimens were also compared with the herbarium records of Department of Plant Resources, Royal Botanical Garden, Godawari, Nepal.

Reagents

All the reagents used in the experiments were purchased from Sigma Co. (USA) and Fisher Scientific (USA). The organic solvents used for the extraction and the chromatography were redistilled using a wire spiral packed double distilling apparatus (Normschliff Geratebau, Wertheim, Germany) and Milli-Q water that was generated through a water purification system (Millepore Corporation, Bedford, USA).

Phytochemical screening

The plant samples were grinded in a blender (MR 350CA, Braun, Spain) and used for the phytochemical screening test. Chemical testes were carried out on the aqueous and alcoholic extracts using standard procedures to identify the constituents as described in literatures [7-10].

Volatile compounds

Extraction of volatile organic compounds

Fifty grams of sample were homogenized in a blender (MR 350CA, Braun, Spain) and mixed with 1 L of distilled water. After adjusting the pH at 6.5 with 1% NaOH, 1 μL *n*-butylbenzene was added as an internal standard. The resultant slurry was used for extraction of volatile organic compounds with 200 ml redistilled *n*-pentane:diethylether (1:1, v/v). The extraction of volatile organic compound was carried out for 2 h, using simultaneous distillation-extraction (SDE) apparatus of Nikerson and Likens [11] type as modified under atmospheric pressure by Schultz *et al* [12]. The solvent, containing compounds extract, was dehydrated for 12 h using 10 g anhydrous Na_2SO_4 and then concentrated to approximately 1.5 mL using the vigreux column. This concentrate was further concentrated to 0.5 mL under gentle stream of N_2 gas and used for gas chromatography-mass spectrometry (GC/MS) analysis.

Chromatographic analysis

Chromatographic analysis was carried out using a Shimadzu GC/MS (QP-5000, Shimadzu Co., Kyoto, Japan) in EI mode. The ionization voltage was 70eV and temperatures of ion source and injector were 230℃ and 250℃ respectively. The capillary column used was a DB-WAX (60 m, × 0.2 mm, i.d., 0.25

μm, film thickness; J&W, USA). The oven temperature programmed at 40 ^{o}C (Isothermal for 3 minutes) was ramped to 150℃ at 2℃/min and to 220℃ at 4℃/min (20 min) followed by 230℃ at 5℃/min. Helium was used as the carrier gas at a flow rate of 1 mL/min, with injector volume of 1 μL using 1:20 split ratio.

Identification of volatile organic compounds
Qualitative analysis of volatile compounds was carried out by identification of compounds from mass spectra with the aid of mass spectral data books. The spectrum of each analyzed volatile compound agreed with that present in the mass spectrum library of WILLY 139, NIST 12 and NIST 62. The content of the volatile flavor compounds was calculated on a dry weight basis by comparing with peak area percent of the internal standard. The mass spectrometer scanned was ranged from 41 to 450 *m/z.*

Biological activities
Extraction of herb
Plant extracts were prepared using Accelerated Solvent Extractor (ASE 200). Total 50 g of samples were weighted and placed in the extraction cells in the oven of the instrument. Extraction was carried out at the temperature of 100 ^{o}C using methanol as extraction solvent. After the injection of the solvent into the cell, a pressurized static extraction phase lasting 5 min was carried out. After removal of the extracts (approx. 20 mL in each cell), they were filtered through a 0.45 µm filter (Waters Millipore). The filtrate was evaporated to dryness using Rotavapour Apparatus (Buchi, Switzerland). Total 12.5 g and 10.25 g of MeOH extract obtained form the 50 g of *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz respectively.

Determination of uterine smooth muscle cells contraction
The PowerLab/4SP electroculograph is a data acquisition and analysis system was used for research. Uterine smooth muscle tissues were obtained from non-pregnant rats. The uterus of the rat was dissected and cut into 10 mm ring segments and placed immediately in Kreb's solution. The Kreb's solution had been cooled previously at 4°C and aerated with carbogen (95% oxygen and 5% carbon dioxide) to maintain a pH of 7.4. The uterine ring segments were suspended in organ bath filled with Kreb's solution. Each ring was suspended under an initial load of 2.0 g in 100 ml organ baths containing Kreb's solution, temperature controlled at 37°C and continuously gassed with carbogen. The tissues were allowed to equilibrate for 1 h with Kreb's solution washing every 10–15 min. After spontaneous uterine contractile activity had been accomplished, plant extracts were added cumulatively to the bath. Changes in isometric force were measured continuously with a channel recorder (Model 79 F Polygraph; Grass Inst., Quincy, MA, USA) which was connected with preamplifier (7P5B, Grass Instr).

Results and Discussion
The phytochemical screening revealed the presence of medicinally active secondary metabolites in both of the species but their concentrations were variable (Table 1). Flavanoids and saponins were found in remarkable amount in both of the samples. The presence of saponins in *Dipsacus* species has been reported [13-14] which is in agreement with our results. It should be noted that steroidal saponin compounds are of importance and interest due to their relationship with such compounds as sex-hormones [15-16]. This could be one of the reason why the above plants are of vegetable for breast-feeding mothers to ensure their hormonal balance. Saponin bearing plants have hemolytic and anti-lipiemic activities and capacity to lower serum cholesterol levels can be considered to be their important characterstics [17]. Similarly, flavonoid rich species can play the role in pharmacological activities as anti-inflammatory, analgesic, anti-oxidant, antifungal and immunostimulant providing a key role of flavonoids to their biological actions [18]. Beside the flavonids and saponins, some other phytochemical groups were also detected in both of the samples. It is known that the plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are

generally superior in medicinal activity as well as exhibit physiological activity. Most important bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds [19], those we found in our samles. Phytochemical screening of samples showed that both of the plants can stand for the medicinally important constituents. There was definite co-relation between the traditional application of plants and possession of secondary metabolites, which supports the scientific basis for the traditional medicinal system. This result may serve for future workers to select a group of plants having similar chemical constituents to isolate biologically active principle or to prepare remedies for particular case.

Table 1. Chemical Constituents of Some Species of Nepalese Medicinal Plants

S.N.	Plant name	Alkaloids	Flavonoids	Glycosides	Saponins	Tannins	Terpenoids
1	*Dipsacus mitis* Wall.	-	++	-	+++	-	+
2	*Woodfordia fructicosa* (L.) Kurz	++	+++	+	++	++	+

If the PPT is slight : +, Medium : ++, Heavy :+++, Not : -

The volatile compounds of *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz were extracted by solvent extraction (P:E,1:1) method for 2 h using SDE apparatus and analyzed by GC-MS. Compound 2-butenal (8.77%) α-terpineol (6.52%), hexanal (6.46%), hexanol (5.59%), 1,8-cineole (5.33%), 2-heptanol (4.90%), linalool (4.44%) were detected as the major constituents of *Dipsacus mitis* D. Don while compounds furfural (11.05%), linalool (8.04%), 3-methyl butanal (6.76%), heptadecanone (5.40%), α-terpeniol (4.96%), undecanoic acid (3.89%) were detected as the major constituents of *Woodfordia fruticosa* (L.) Kurz. Result showed that rhizomes of *Dipsacus mitis* D. Don contained 61.93 mg/kg volatile compounds and *Woodfordia fruticosa* (L.) Kurz contained 224.98 mg/kg volatile compounds (dry weight basis). Total 53 volatile compounds were tentatively identified from *Dipsacus mitis* D. Don and 78 were identified from *Woodfordia fruticosa*. Alcohols were dominant in both the plants, *Dipsacus mitis* D. Don accounting with 45% and *Woodfordia fruticosa* (L.) Kurz accounting with 32%.

Table 2. Relative content of functional groups of volatile organic compounds identified in *Dipsacus mitis* D.Don

No.	Functional group	*Dipsacus mitis* D.Don.		*Woodfordia fruticosa* (L.) Kurz	
		Area (%)	Number of compounds	Area (%)	**Number of compound**
1	Acid	6.10	4	17.01	9
2	Alcohol	45.77	21	32.20	24
3	Aldehyde	29.64	14	30.44	15
4	Ester	2.95	2	2.88	4
5	Furan	2.56	3	1.48	3
6	Hydrocarbon	-	-	5.39	9
7	Ketone	8.62	6	9.30	8
8	N-Compound	4.21	3	1.30	6
	Total	**100**	**100**	**100**	**77**

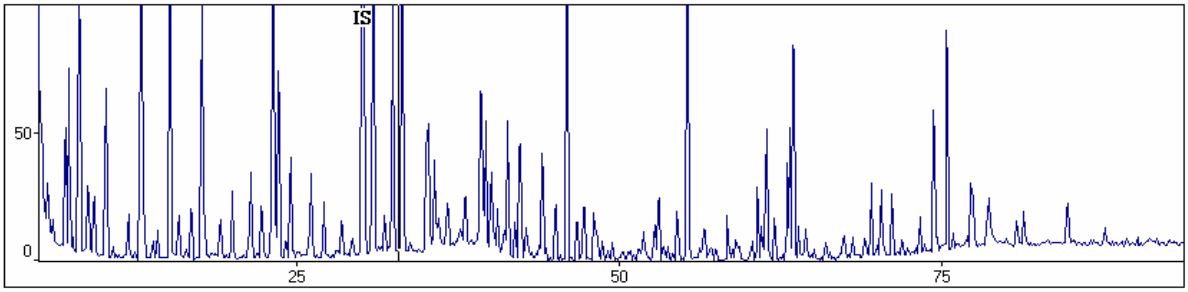

Figure 1. GC/MS total ion chromatograms of volatile components in *Dipsacus mitis* D.Don.

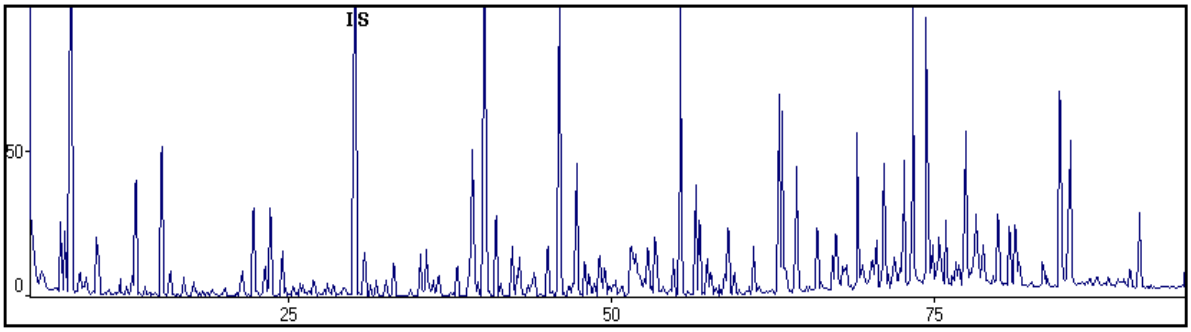

Figure 2 . GC/MS total ion chromatogram of volatile organic compounds of *Woodfordia fructicosa* (L) Kurz extract.

Numerous bioactive volatile compounds were detected in these herbs and their characteristics are discussed in this paper. The most abundant compound furfural has a wide variety of uses such as weed killer, fungicide, affects yeast survival and also affects biochemical enzyme activities [20]. Linalool, a major compound of this oil, is known for various pharmacological activities as well as it is important substance used in foodstuffs as a food additives [21]. The compounds linalool, α-terpineol, geraniol were major oxygenated monoterpenes while farnesol, [*Z*]-Nerolidol were the major oxygenated sesquiterpene. Monoterpenes are generally regarded as safe substances. α-Terpineol is probably the most important of the monocyclic monoterpene alcohol possessing various biological activities and flavor compositions [22]. 4-Terpinenol, which occurs in appreciable amounts in this oil, is also reported to show activity against the microorganisms [23]. Geraniol, exerts anti-tumor activity against various cancer cells both in vitro and in vivo [24]. Consequently monoterpene phenols were previously reported to be active against fungi [3] and can be used as alternative sprout inhibitors [25]. Pharmacologically active some compounds related to hydrocarbon sesquiterpenes such as (*E*)-caryophyllene, α-humulene were detected in this study which have been claimed to contain the antibacterial, antifungal or antioxidant properties [26]. Plant oils containing monoterpene hydrocarbons including β-Myrcene are used as flavoring additives in foods and beverages, as fragrances in cosmetics, and as scent in household products [27]. Safrole has been used as a topical antiseptic and it is carcinogenic to the liver so it is no longer used as a flavoring agent in foods. Farnesol is a precursor of vitamin E and K1, modulates cholesterol synthesis is metabolized to steroids in retina [28]. The presence of pyrazine compounds in many plant species results from Maillardtype non-enzymetic reactions between reducing sugars and free amino acid or amide. The pyrazine compounds impart a reportedly nut-like aroma. Compounds α-terpineol, linalool, furfural and geraniol were common in both herbs.

Table 3. Volatile Organic Compounds of *Dipsacus mitis* D.Don. and *Woodfordia fruticosa* (L.) Kurz

No.	RI[b)]	Compound Name	*Dipsacus mitis* Content (%)	*Woodfordia fruticosa* Content (%)
1	850	Butanal	1.30	-
2	862	Ethyl acetate	2.48	0.81
3	871	2-Methylbutanal	0.03	2.84
4	878	2-Butanone	-	0.70
5	896	3-Methylbutanal	3.14	6.76
6	922	Ethanol	0.98	0.26
7	938	2-Ethylfuran	0.60	0.21
8	967	2,3-Butanedione	-	0.64
9	969	2-Pentanone	3.05	-
10	970	Pentanal	-	0.53
11	1036	2-Butenal	8.77	0.28
12	1040	2,3-Dihydrofuran	0.89	-
13	1041	4-Heptanone	-	1.34
14	1079	Hexanal	6.46	1.73
15	1090	2-Methylpropanol	0.53	0.26
16	1107	3-Pentanol	0.50	-
17	1121	2-Pentanol	2.66	-
18	1124	[*E*]-2-Pentenal	0.50	-
19	1146	Butanol	0.27	0.09
20	1161	β-Myrcene	-	0.15
21	1179	2-Heptanone	0.16	0.19
22	1181	Pyridine	1.05	-
23	1193	Heptenal	0.56	0.27
24	1206	1,8-Cineole	5.33	-
25	1207	2-Methylbutanol	1.59	0.33
26	1213	2-Hexenal	2.11	1.10
27	1228	2-Pentylfuran	1.07	0.47
28	1252	Pentanol	1.12	0.33
29	1264	Methylpyrazine	-	0.19
30	1279	α-Terpinene	-	0.10
31	1285	Octanal	0.41	0.10
IS	*1310*	*Butylbenzene*	0.00	-
32	1321	2-Methyltetrahydrofuran	-	0.80
33	1322	2-Heptanol	4.90	-
34	1328	2,3-Dimethylpyrazine	-	0.12
35	1335	6-Methyl-5-hepten-2-one	0.36	0.19
36	1344	2-Octanol	3.09	-
37	1356	Hexanol	5.92	0.43
38	1382	4-Ethylpyridine	1.51	-
39	1384	3-Hexenol	1.67	0.53

40	1390	Nonanal	1.05	0.67
41	1398	Tetradecane	-	0.15
42	1403	Trimethylpyrazine	-	0.31
43	1405	2-Hexenol	0.39	-
44	1427	5-Methylhexanol	-	0.36
45	1446	[*Z*]-Linalool oxide		3.33
46	1446	Acetic acid	2.70	-
47	1452	3-Decanone	-	0.21
48	1452	1-Octen-3-ol	1.40	-
49	1459	Furfural	1.00	11.05
50	1473	[*E*]-Linalool oxide	-	1.02
51	1477	4-Ethyenylpyridine	1.66	-
52	1478	Tetramethylpyrazine	-	0.12
53	1491	[*E,E*]-2,4-Heptadienal	1.24	-
54	1492	2-Ethylhexanol	1.57	0.90
55	1500	2-Acetylfuran	-	0.63
56	1510	Pyrrole	-	0.19
57	1518	Benzaldehyde	1.45	-
58	1519	Benzaldehyde	-	0.36
59	1535	2-Nonenal	-	0.67
60	1535	Octanol	0.54	-
61	1549	Linalool	4.44	8.04
62	1569	5-Methylfurfural	-	2.13
63	1569	3,5-Octadien-2-one	0.48	-
64	1580	Linalool acetate	0.47	-
65	1583	[*E,Z*]-2,6-Nonadienal	-	0.22
66	1595	β-Caryophyllene	-	0.52
67	1602	4-Terpineol		0.33
68	1625	Butanoic acid	-	0.10
69	1636	Benzeneacetaldehyde	-	1.73
70	1657	Furfuryl alcohol	-	0.73
71	1662	Nonanol	0.77	-
72	1666	3-Methyl butanoic acid	-	1.55
73	1689	α-Humulene	-	0.48
74	1697	*α*-Terpineol	6.52	-
75	1698	α-Terpineol	-	4.96
76	1720	Junipene	-	1.71
77	1725	Caryophyllene	-	1.15
78	1736	Pentanoic acid	-	0.63
79	1761	[*Z,E*]-α-Farnesene	-	0.28
80	1765	Epoxylinalol	-	0.89
81	1773	Methyl salicylate	-	0.28
82	1799	Nerol	-	0.64

83	1809	[*E,E*]-2,4-Decadienal	1.67	-
84	1844	Hexanoic acid	1.33	2.87
85	1849	[*E*]-Geraniol	1.29	2.33
86	1856	[*E*]-Gerayl acetone	1.99	-
87	1874	Safrole	-	1.79
88	1876	Benzyl alcohol	0.36	0.57
89	1912	Benzeneethanol	-	0.85
90	1951	Heptanoic acid	-	0.80
91	1974	2-Acetylpyrrole	-	0.37
92	1995	[*Z*]-Nerolidol	-	2.23
93	2038	Farnesol	-	0.43
94	2055	Octanoic acid	0.54	1.47
95	2111	Heptadecanone	-	5.40
96	2130	Nonanoic acid	1.53	3.64
97	2134	Eugenol acetate	-	1.06
98	2148	3-Methoxyacetophenone	2.60	-
99	2150	Tetradecanol	-	0.56
100	2157	Methyl nonanoate	-	0.74
101	2184	Decanoic acid	-	2.06
102	2197	Docosane	-	0.85
103	2373	Undecanoic acid	-	3.89
		Total	**100**	**100**

We studied the effect of the different plant extracts on the smooth muscle strips from rat uterus. The effects of increasing concentrations of the extracts on the amplitude and frequency of spontaneously contracting uterine tissues were tested. Dramatic muscular relaxation on spontaneous contractility was obtained at a concentration of 6500 μg/ml of *Dipsacus mitis* D. Don slight relaxation on spontaneous contractility was obtained by methanol extract of *Woodfordia fruticosa* (L.) Kurz upto concentration of 20000 μg/ml. Frequency of contraction in both cases was decreased as dose of drug was increased. The inhibition of Kreb's solution induced contraction could be due to an effect on one of the components of the vessel wall, namely the endothelium, the smooth muscle, or the extracellular matrix.

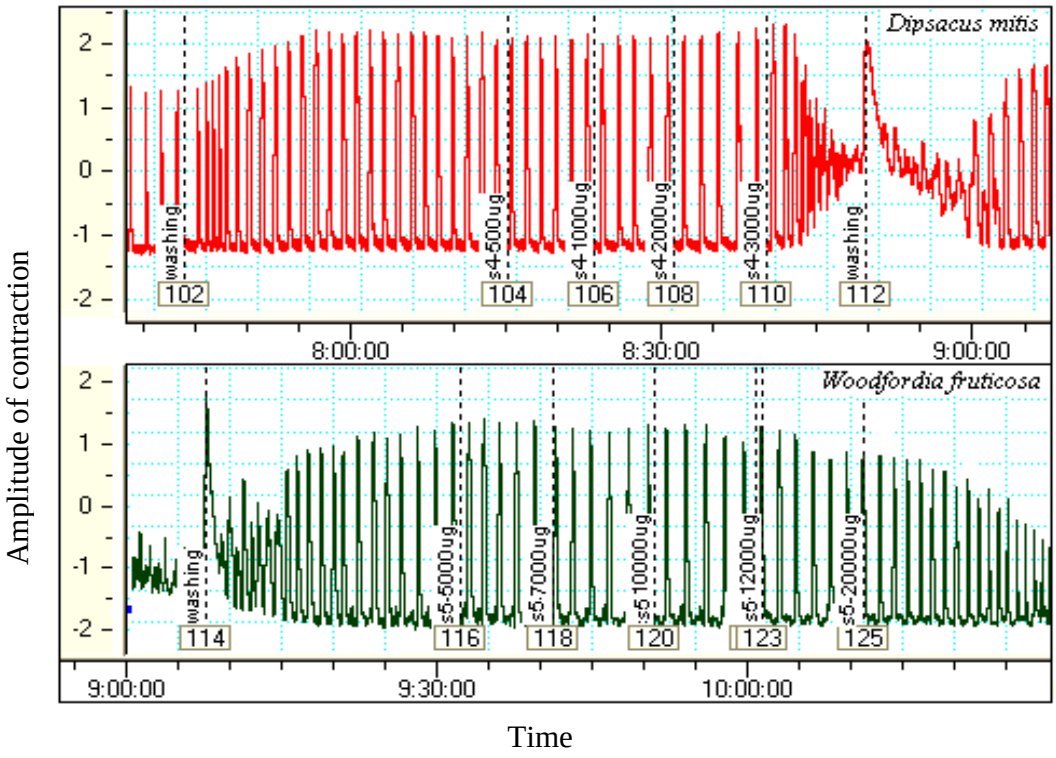

Fig. 3. Effect of methanol extracts of *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz on uterine smooth muscle tissues of non-pregnant rat

After loading the tissue, the system was operated for 30 minutes in Krab's solution to check and maintain contractibility. Normal Kreb's solution caused a significant contraction that reached a maximum within few minutes. Kreb's solution contains numerous nutrituents such as NaCl, KCl, $MgCl_2$, KH_2PO_4, $NaHCO_3$, $CaCl_2$ and Glucose. These nutrituents made cell active and could be seen on polygraphs. The observed inhibition of vascular contraction after adding MeOH extract was result of an effect of plant secondary metabolites. Effective weight 6,500 μg of plant is equivalent to 317 mg of its raw weight and 20,000 μg equivalent to 975 mg of its raw weight. This result appears to justify their traditional uses and give additional interest that these plants could be useful for controlling the preterm birth problem. For further confirmation and accurate report, dose dependent steps, in-vivo and in-vitro tests, ion-channel studies and more pre-clinical studies should be done. We concluded that inhibited spontaneous induced uterine smooth muscle contraction by MeOH extract of *Dipsacus mitis* D. Don and *Woodfordia fruticosa* (L.) Kurz justify their traditional uses. *Dipsacus mitis* D. Don is possesses the least relaxation effect in rat uterine smooth muscles.

Acknowledgement

I would like to acknowledge to Prof. Kim Kyong-Su, Department of Food and Nutrition; and Prof. , Song Chang-Hun, Department of Obstetrics and Gynecology of Chosun University, Republic of Korea for the kind guideline and research facility.

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