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A preliminary report on the galactagogic attributes of some plants used by tribal of two villages of West Midnapore district, India

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Abstract
Development of young ones depends on increasing food intake, digestibility, stimulating of the immune system, and increasing milk yield. These are some of the conditions which is indeed necessary for the ultimate survival of all young animals. However, it has been observed that milk production decreases in lactating/nursing mother which is due to several factors such as stress, disruption of endocrine organs, more or less hormone secretion etc. There are numerous synthetic galactagogues but their efficacy has never been tested. Throughout the world, women of different ethnic backgrounds often select different approaches according to their ceremonial or experience to increase their milk production such as following special diets, use of herbal or natural substances. These suppositories are often used singly or in combination which are prescribed by village elderly or quacks and lacks the scientific basis. Therefore, the present investigation was carried out in two villages of West Midnapore district, India to know the traditional knowledge which would lead to the development of ethno-botanic resources for inexpensive galactagogues drugs and their scientific authentication.

Keywords: West Midnapore, plants, attributes, two villages

Introduction
Milk supply is essential for the new born baby and milk supply depends on several maternal hormone secretion and these hormones include oxytocin which initiates and stops milk supply. Prolactin is another important hormone in this regard which also initiates and stimulates milk secretion [1-2]. Milk secretion is a complicated process because it depends on many factors viz. periodic removal of milk, emotion and stress. Removal of milk from the breast depends on Milk Ejection Reflex (MER) which in turn helps in the release of the hormone oxytocin. It is well known that lactogenesis is inhibited during pregnancy due to presence of placental steroids despite high levels of prolactin in blood, also during pregnancy alveoli and secretory ducts develop due to presence of oestrogen and progesterone [3-5].

Breast feeding is quite ineffective in urban areas because either the baby or new born cannot suckle properly or there may be some hormonal imbalance in the mother or if there is a problem in the breast tissue. Further, many mothers of urban setting often believe that suckling can alter the shape and size of breast. As lactation primarily depends on hormone action which in turn also depends on emotion and stress so control of stress can also effectively help in lactogenesis [6-7].

Plants are the richest source of drugs in traditional systems of medicine therefore human has been using plants extracts to protect from various ailments. Various phyto-constitutents present in medicinal plants such as alkaloids, flavonoids, tannins, terpenes, amino and inorganic acids have a profound effect in curing various ailments [8-9]. Tribal population in India constitutes about 8.6% of the total population of the country and majority of these tribes live in small habitats in remote areas with no proper access to education, healthcare system [10]. They are considered to be weakest sections in terms of socioeconomic status and their socio-cultural pattern also varies from tribe to tribe and region to region. West Midnapore District of West Bengal is predominantly inhabited by tribal communities and the major tribal communities are Santhals, Bhumij, Munda, Lodha, Kora and Mahali. The literacy rate among these tribes was low however, recently there is a slight rise in literacy rate due to some effective steps of the state government. Since most of these populations do not have right access to proper health care system they still depend on the old belief of obtaining nutrition from plants. These drugs are mostly prescribed without knowing their biologically active compounds. The present review was done after obtaining information from the old tribal villagers with the objectives to describe the main medicinal plants used as galactagogues which are predominantly used by these tribal communities. In fact, the present review can also

serve as a useful and effective tool for the selection of medicinal plants with immense potential for the research for novel galactagogues and also lead to the development of ethno-botanic resources for inexpensive galactagogues drugs and their scientific validity. Further, the present investigation was also undertaken with a view to provide a comprehensive account of potential medicinal plants as galactagogues used by tribal in the study area.

Methodology

Several surveys were undertaken in the blocks of West Midnapore district during the period of 2016 in the village of Rakhalmara which is located in Jamboni block. The village has an area of 1.9 square kilometers and comprises mostly of ST population while Metial village which comes under the jurisdiction of Jhargram district comprises of both SC, ST and general caste population. Data collection was done through interviews and discussion with elderly people comprising both male and female members and tribal practitioners or quacks. These villagers have limited access to dispensaries and hospital because they live in isolated areas and predominantly depend on the elderly persons and quacks for their primary medication. Additional information regarding the dosage, part of the plants used, whether they are used alone or in combination with other plants, form in which they are advised to administer were also recorded.

Further attempts were made to know what compounds are present in few plants by using GC-MS analysis. The chemical composition of the plants extracts was investigated through Gas Chromatography-Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436-GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30mm; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program was as follows: 110 °C hold for 3.5 min, Up to 200 °C at the rate of 10 °C/min-No hold, Up to 280 °C at the rate of 5 °C/min-with 9 min hold, Injector temperature 280 °C and total GC running time was 37.5 min. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan interval of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 290°C, source temperature 250 °C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8 and compared with NIST Version 11.0 library database of National Institute Standard and Technology (NIST) which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and peak area of the components of the test materials were ascertained.

Results and Discussion

During our study we found that tribal medicine men or quacks were initially reluctant to share their knowledge about these medicines. They have a notion that if they disclose the identity of the plant then it would lose its pharmacological potential. Hence, we have to approach them frequently (twice a month) and gather their confidence. Further, these tribal medicine men did not divulge the identity of the plants to the patients or to their descendants because they feel a professional competitor would harm his identity, status and recognition in the adjacent areas. In these two villages most of the mothers find it difficult to produce adequate milk since they come from low socioeconomic status and they cannot procure rich nutritious diet and therefore they depend on the village elderly people, traditional practitioners and local quacks for enhancing their milk production. We came across several women who assist their husbands in fields even during pregnancy apart from regular cooking and managing their families. They even spend hours for collecting woods and often travel 3-5 kilometers a day. It would be subtle to say that all these phenomena conjointly might be responsible for decrease in milk production in most of these village women. Few plants which are commonly used by these tribal villagers have been proven scientifically by different investigators worldwide, however some plants have no data neither literature available in various biological databases about their attributed property. Table 1 depicts several plants commonly used by these villagers and their mode of usage.

Phytosterols are plant sterols found in plants and have a structure similar to cholesterol and used as precursor hormones and reproductive growth. GCMS is one of the hyphenated analytical techniques that help to do qualitative and quantitative evaluation of the chemical components in a sample. It involves two different techniques in a single method. It works on the principle of gas chromatography (GC) and mass spectrometry (MS). By GC, the components of a mixture are separated depending on their volatility (ease with which they evaporate into gas) and by MS individual characterization of the components are done depending on their structure. β-sitosterol is a phytoestrogenic compound may be responsible for the increase in lactation in females. Here we present some ethanolic extracts of the plants which are used by these tribal populations (Table 2-4) and the phytoconstituents which might be responsible for galactagogic property.

There are over 400 different tribes in India which constitute about 8 % of India's population. Tribal societies have discovered treatment of ailments through trial and error basis and utilize the resources around them to optimum. Small survey such as these reveals that the plants which are used to increase lactation have been proven but there is dearth/lack of supporting data for their attributed property. Some plants have high flavonoids and alkaloids which might interfere positively with the endocrine system and hormones which results in milk flow. Also, the phytoestrogenic compounds present therein might stimulate mammary gland to stimulate milk production. There are few reports on human trial which has not been conducted systematically [11-13] though there are some experimentation carried on animal models [14-16]. It is important that more research is conducted in this area including their use, safety and efficacy studies because exposure to modernization and lack of interest in tribal population may lead to depletion of traditional knowledge and its uses in time course [14-16]. In recent years, ethno medicinal studies received considerable attention as this brings to light the numerous unknown medicinal aspects especially from plant origin which needs evaluation on modern scientific lines such as phytochemical analysis, pharmacological screening and clinical trials. Efforts are on to know the phyto-constituents present which might be responsible for increase

in milk production. More such studies are encouraged to confirm or disprove our findings.

Table 1: Lists of some plants commonly used by tribal of two villages of West Midnapore district. *indicates plants for which GC-MS was conducted

Sl. No.	Scientific Name	Family	Local Name	Uses
1.*	*Bauhinia variegata*	Fabaceae	Sada Kanchan, Kochnar	Flowers are made into paste and applied on breast
2.*	*Pergularia daemia*	Asclepiadaceae	Dudhi	The leaves and stem of the plant are routinely used to feed farm animals for increasing milk production especially after parturition for about a month, but there are no reports on human consumption.
3.	*Vitex negundo*	Verbenaceae	Nishinda, Lunguni, Sindhuhari	Seeds paste are consumed by mother, even leaf paste are applied on breast to stimulate milk production, dried leaves made into powder and taken with cold water for seven days continuously after parturition.
4.	*Opuntia stricta*	Cactaceae	Nagphani, Nagphini	Warm watery flower extract is applied regularly on breast
5.	*Chlorophytum arundinaceum*	Liliaaceae	Sada Musli	Root paste is taken with milk for 3 to 4 days
6.	*Ricinus communis*	Euphorbiaceae	Digherandi	Paste of leaves are placed on cloth and tied on the breasts for 4 to 5 hours
7.	*Hemidesmus indicus*	Asclepiadaceae	Anantamul, Palumala, Ladugra	Females consume roasted roots in turmeric leaves and few cumin seeds
8.	*Curcuma longa*	Zinziberaceae	Halud	Sundried rhizome powder mixed in warm rice taken twice daily after birth of new born for 15 to 20 days
9.	*Amaranthus spinosus*	Amaranthaeceae	Katanotey, Lal bhiji ara	Fresh roots cleaned with warm water are chewed by nursing mothers daily early in morning; roots are tied on the left arm with a black thread of expected mothers.
10.	*Ficus hispida*	Moraceae	Kakdumur, Kotang	Ripe fruits are chewed by mothers to enhance lactation, often the farm animals are fed regularly after giving birth to young ones, Boiled green fruits are given to mothers as galactagogue
11.	*Solena amplexi caulis*	Cucurbitaceae	Rakhaal sosa, Banleundari	Leaves and root decoction is used to enhance milk yield or even eaten raw
12.	*Cyamopsis tetragonoloba*	Fabaceae	Guar	Beans of the plants are roasted and then crushed and taken 3 to 4 days a week.
13.	*Moringa oleifera*	Moringaceae	Sojne, Mung-ara, Munga	Flowers are made into a paste and applied on breast and the flowers are cooked and consumed everyday
14.	*Asparagus racemosus*	Liliaceae	Gaisira, Satavari, Finajapari	Sundried root is made into powder and taken with sugar or salt as per the choice/liking of the mother
15.*	*Butea superba*	Fabaceae	Latapalash, Lara murud, Nari murup	Leaf paste and stem paste are applied on breast and tied with a cotton cloth for 4-5 days after parturition
16.	*Holarrhenna pubescenns*	Apocynaceae	Kurchi, Patadali	Leaf paste are applied thrice a week on the breast to increase lactation
17.	*Musa paradisica*	Musaceae	Kola	Flower/ Florets were isolated dried, crushed and taken orally every day for about a month.
18.	*Trigonella foenum graecum*	Fabaceae	Methi	Fresh seeds are made into paste and then consumed before any food is taken early in morning.
19.	*Bixa orellana*	Bixaceae	Latkan	Seeds are made into paste and applied locally

Table 2: GC-MS analysis of the ethanolic extract of *Pergularia daemia*, showing phytoconstituents. RT-retention time.

No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area
1.	8.04	Trans-13-Octadecanoic acid	$C_{18}H_{34}O_2$	282	0.51
2.	8.56	2-Hexadecanol	$C_{16}H_{34}O$	242	1.60
3.	8.71	9-Hexadecanoic acid	$C_{16}H_{30}O_2$	254	0.57
4.	10.17	2-Pentanone, 3,3,4,4-tetramethyl	$C_9H_{18}O$	142	0.29
5.	11.03	Diethyl Pthallate	$C_{12}H_{14}O_4$	222	2.84
6.	11.35	E-9-Tetradecanoic acid	$C_{14}H_{26}O_2$	226	0.63
7.	13.44	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	1.47
8.	13.57	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356	0.84
9.	14.29	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376	8.90
10.	15.52	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	3.35
11	16.04	Coumatetralyl isomer-2ME	$C_{20}H_{18}O_3$	306	4.48
12.	16.81	4-Oxazolecarboxylic acid, 4,5,-didyhdro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	11.85
13.	17.02	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	2.02
14.	23.80	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	16.46
15.	35.58	l-Monolinoleoylglycerol trimethyllsilyl ether	$C_{27}H_{54}O_4Si_2$	498	1.85
16.	37.25	β-sitosterol	$C_{29}H_{50}O$	414	1.47
17.	37.42	α-Amyrin	$C_{30}H_{50}O$	426	0.51
18.	38.27	β-Amyrin	$C_{30}H_{50}O$	426	0.36

Table 3: GC-MS analysis of the ethanolic extract of *Bauhinia variegata*, showing phytoconstituents. RT-retention time.

No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area
1.	8.03	2-Butyl-2, 7 octadien-1-ol	$C_{12}H_{22}O$	182	3.01
2.	8.37	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228	6.05
3.	8.94	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3α, 5aα, 9α, 9aα)]	$C_{15}H_{26}O$	222	0.94
4.	13.44	Tert-Hexadecanethiol	$C_{16}H_{34}S$	258	3.56

5.	13.95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	9.56
6.	14.27	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252	2.25
7.	14.50	9,12-Octadecadien-1-ol(Z,Z)-	$C_{18}H_{34}O$	266	2.67
8.	14.90	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$	304	1.54
9.	15.10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.47
10.	15.50	Dibutyl pthallate	$C_{16}H_{22}O_4$	278	1.02
11	16.00	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	9.89
12.	17.38	[1,1-Bicyclopropyl}-2octanoic acid, 2 hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	2.27
13.	17.48	9-Octadecenoic acid (Z) methyl ester	$C_{19}H_{36}O_2$	296	4.30
14.	17.64	Phytol	$C_{20}H_{40}O$	296	12.30
15.	18.43	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	0.23
16.	18.44	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.12
17.	18.94	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.71
18.	23.77	Cyclopropaneoctanoic acid, 2-[(2-pentylcyclopropyl)methyl]-, methyl ester, trans-	$C_{21}H_{38}O_2$	322	3.17
19.	28.04	Squalene	$C_{30}H_{50}$	410	9.80
20.	37.28	β-sitosterol	$C_{29}H_{50}O$	414	5.15

Table 4: GC-MS analysis of the ethanolic extract of *Butea superba*, showing phytoconstituents. RT-retention time

No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area
1.	6.92	Phenol, 2 methyl-5-(1methylethyl)-	$C_{10}H_{14}O$	150	4.29
2.	8.37	Dodecanoic acid, 3 hydroxy-	$C_{12}H_{24}O_3$	216	3.62
3.	11.05	Octanal, (2,4-dinitrophenyl)hydrazone	$C_{14}H_{20}N_4O_4$	308	1.81
4.	11.96	α-D-Mannofuranoside, 1-O-decyl	$C_{16}H_{32}O_6$	320	1.56
5.	13.94	3,7,11,15-Tetramethyl-2-hexadecen-1ol	$C_{20}H_{40}O$	296	0.87
6.	14.27	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$	304	7.81
7.	14.88	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	2.28
8.	15.10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.17
9.	15.51	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	9.50
10.	16.15	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.50
11	17.64	Phytol	$C_{20}H_{40}O$	296	12.13
12.	28.35	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3β)-	$C_{31}H_{50}O_3$	470	3.57
13.	29.56	Androstane-11,17-dione, 3-[Trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime] (3α, 5α)-	$C_{29}H_{43}NO_3Si$	481	7.63
14.	37.21	β-Sitosterol	$C_{29}H_{50}O$	414	1.26

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