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Phenolics from *Phaleria nisidai* with Estrogenic Activity

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Abstract: The methanol extract of *P. nisidai* leaves yielded a benzophenone rhamnoside, iriflophenone 2-*O*-*α*-L-rhamnopyranoside (**1**) in addition to genkwanin 5-*O*-*β*-D-primeveroside (**2**) and mangiferin (**3**). The isolated compounds as well as the derived aglycones of **1** and **2** assigned as compounds **4** and **5,** respectively, were tested for their estrogenic activity on ER*α* using an estrogen receptor competitive binding screen. Compounds **1**, **4** and **5** showed almost the same binding ability to ER*α* with IC_{50} of 630 *μ*M, 700 *μ*M and 800 *μ*M, respectively. Virtual docking with ER*α* revealed that compound **1, 4** and **5** strongly hydrogen bond with amino acids Glu353, Arg349, Gly521 and His524, in the estrogen receptor ligand binding domain, similar to that of mammalian estrogen 17*β*-estradiol.

Keywords: *Phaleria nisidai;* estrogenic activity; ER; benzophenone; mangiferin.

1. Plant Source

P. nisidai leaves were collected in bulk from three different sites on the Palauan islands and sites were marked using GPS coordinates. Local botanist, Ann E. Kitalong, identified plants and voucher specimens were stored in the Belau National Museum Herbarium. The bulk *P. nisidai* leaves were then air dried under non-UV conditions and then placed in light drier over night. The leaves were then shipped, in moisture absorbing packaging, to Toyama University, Sugitani Campus, after passing Palau Bureau of Agriculture Quarantine under research permit no: BOA04-05.

2. Previous Studies

Palauan folk medicine has been a cornerstone for the survival of the secluded island population for centuries. As a result of the rather minute population of this equatorial island there have been few, if any, scientific studies on the efficacy of Palauan herbal medicines.

Leaves from *Phaleria nisiddai* (Kaneh.) family Thymelaeaceae has been used traditionally for the induction of abortion [1, 2] by consumption of fresh leaves. The leaves have been used as a

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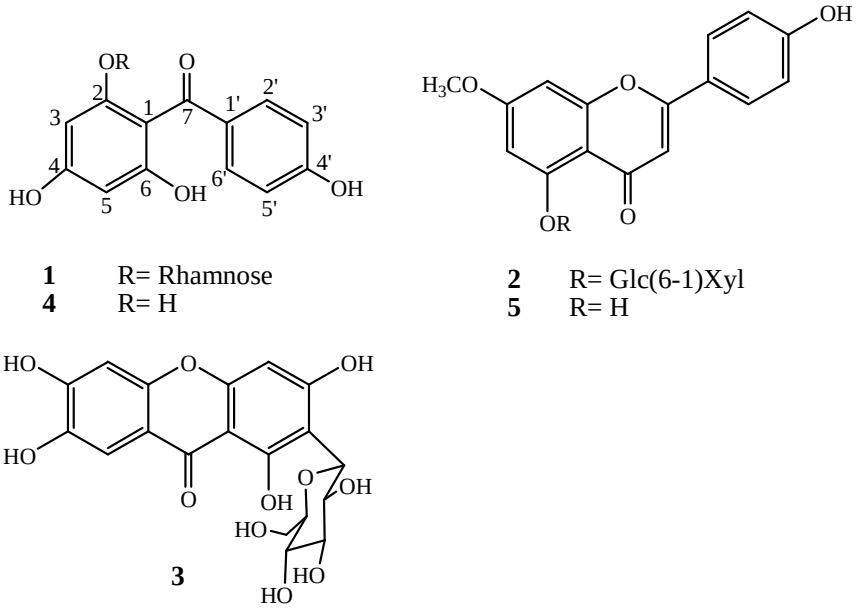
treatment during first birthing ceremonies as well as to induce fertility. In addition, it can be used as a treatment for diarrhea and topically for abscess and bruises. The leaves of *P. nisidai* are also used in many decoctions as energizer and tonic mixed with many other herbal remedies [1, 2]. The vast ethnopharmacological implications of *P. nisidai* are evidenced by the local population changing the original name of the plant from *ongael* to *delal a kar*, literally meaning "mother of medicine". Recent research has also shown its effects as an immunostimulant [3].

There have been few studies on the ethnopharmacology and phytochemistry of Palauan plants and current research may be used to bolster traditional practices on the island and further scientific knowledge of ethno medicine in Micronesia. The objective of this study is to determine the estrogenic activity of this local remedy to validate traditional medicine practices of the Palauan people.

3. Present Study

The 50% methanol fraction of *P. nisidai* yielded three compounds identified as iriflophenone 2-*O*-α-L-rhamnopyranoside (**1**) [4], genkwanin 5-O-β-D-primeveroside (**2)** [5], mangiferin (**3)** [6]. The aglycones of compound **2** and **3** were obtained through acid hydrolysis of their original compounds to obtain compounds **4** and **5**, respectively. Compounds **1**-**5** were tested for their binding ability to ERα. In addition, a docking study was carried out for the active compounds to estimate their mechanism of action on a molecular level.



1 R= Rhamnose
4 R= H

2 R= Glc(6-1)Xyl
5 R= H

3

Figure 1. Isolated compounds from *P. nisidai* (**1**-**3**) and their derived aglycones **4** and **5**.

Estrogenic Activity of the Isolated Compounds; the isolated compounds were tested for their binding abilities to the estrogen receptor (ERα) at 1 mM concentration; compounds with a 50% inhibition of estradiol binding to ERα at this concentration were additionally tested at lower concentrations to calculate their IC_{50}. 17β-Estradiol (E_2) was used as the positive control with an IC_{50} of 18 nM (Table 1). Compounds **1**, **4** and **5** showed significant binding to ERα at IC_{50} of 630 μM 700 μM and 800 μM, respectively. However, compound **1** exhibited 80% inhibition of labeled estradiol binding to ERα at 1 mM. On the other hand, mangiferin weakly competed with 17β- estradiol binding to ERα with an IC_{50} of 1 mM (Table 1)

Virtual Docking Study; it has been established that estradiol fits into ERα through hydrogen bonding with Arg394 and Glu353 with its 3-OH group at ring A, in addition to a hydrogen bond with His524 with 17β-OH group and by fitting with its hydrophobic core into the hydrophobic pocket of ERα (Fig. 2a) [7]. A distance of 10.9Å between the two hydroxyl groups in estradiol was found to be essential for the activity through binding to the right amino acid residues in the estrogen receptor active site [7].

Compound **1** binds to estrogen receptor through a hydrogen bonding with His524 (1.82Å) and Gly521 (1.80Å) through its 4′ hydroxyl in a similar way to 17β-OH group of estradiol (Fig. 2b). Hydroxyl groups at position 4 and 6 bind to Arg394 similar to the phenolic group of estradiol. In addition, the rhamnose hydroxyls bind to Thr347. Compound **4** binds to Glu353 (1.8 Å) and Arg394 (2.2 Å) through its phenolic group at C-4 in a similar orientation to the aromatic ring of esradiol (Fig. 2c). Moreover, the OH groups at C-4′ and C-2 bind to Thr347 and Leu346, respectively. However compound **4** failed to bind to Gly521 and His524 in contrast to from compound **1** and estradiol. Finally, compound **5** binds to Arg394 (2.02Å) and Glu353 (2.37Å) through hydroxyl at C-4′ and to Gly521 by C-5 hydroxyl (Fig. 2D).

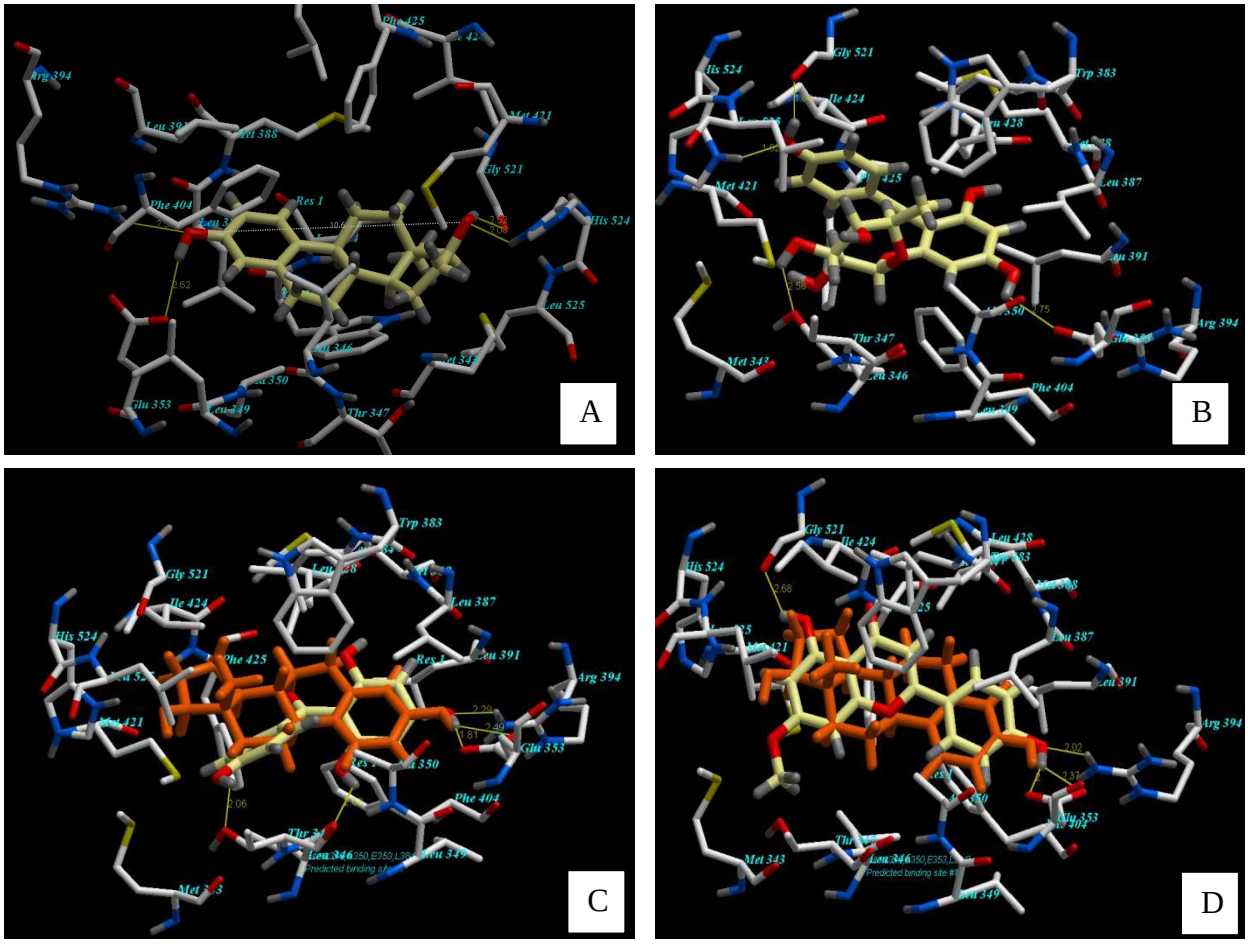


Figure 2. Virtual docking of isolated compounds to ERα active site; (A) estradiol, (B) compound **1**, (C) superimposition of compound **4** (yellow) and estradiol (orange), (D) superimposition of compound **5** (yellow) and estradiol (orange).

The current research reported the estrogenic activity of compounds from *P. nisidai* which is a step in validating the plant traditional medicinal use. The binding ability of compound **1** and its aglycone (**4**) was almost similar. However, virtual docking of both compounds to ERα showed a significant difference in their binding way relative to estradiol which warrants for further investigation of the agonist and/or antagonist action of these compounds. On the other hand, compound **2** revealed lower binding ability to ERα in comparison to the aglycone **5**. The lower activity of compound **2** is mainly due to its bulky diglucoside (primveroside) moiety which prevents binding of C-5 hydroxyl to Gly521 as in its aglycone (**5**). Furthermore, the hydrophilic nature of compound **2** makes the binding of the compound to the hydrophobic pocket of ERα weaker than the more hydrophobic aglycone **5**. The previous findings were further confirmed by calculating the docking energies (score) of the tested compounds (Table 1). Estradiol showed the highest docking score (-77.4), compounds **1**, **4** and **5** showed the best docking scores among the tested compounds which is in accordance with their IC_{50} values. Moreover, compound **2** showed the lowest docking score which interpret its inactivity compared to its aglycone.

17β-Estradiol (E2) is the human endogenous estrogen and known to be the most active estrogen receptor agonist [8]. Although the binding affinity of well known phytoestrogens, such as genistein and daidzein, to estrogen receptors is only 1/1000 to 1/10000 of estradiol, they can compete effectively with estradiol for receptor sites in the human body because their plasma levels are 1000 to 10.000 times the circulating concentration of estradiol [9]. Therefore, compounds **1**, **4** and **5** were considered moderately active compounds with almost 30.000~40.000-folds less activity than that of E2. Finally, the high content of mangiferin and its simple and inexpensive isolation from *P. nisidai* makes it as a valuable commercial source for this compound.

Table 1. Inhibition of fluorescence-labeled estradiol binding to ERα by the isolated compounds and their aglycones

Compound	Inhibition of fluorescence-labeled estradiol binding to ERα		Docking score [b]
	1 mM (%)	IC_{50} (M)[a]	Kcal/mol
1	67.9±1.0	$6.3x10^{-4}$±1.3	-63.1
2	46.1±1.2	$>10^{-3}$	-40.1
3	50±0.7	10^{-3}±1.1	-46.9
4	80.6±0.8	$7x10^{-4}$±2.1	-51.4
5	60.2±1.3	$8x10^{-4}$±0.5	-61.9
Estradiol		1.8×10^{-8}± 0.3	-77.4

[a] IC_{50} is the concentration of compound which can decrease the binding of fluorescence labeled estradiol to ERα by 50%. Data are represented as mean values±SEM. [b] Docking score represents the binding potency of compounds to ER, the highest the negative value the potent the binding to receptor.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

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